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TRANSACTIONS
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THE EXTERNAL MORPHOLOGY OF *LACHNOSTERNA CRASSISSIMA* BLANCH
(Scarabæidæ, Coleop.)[1]

By
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Introduction

The present discussion of the external features of *Lachnosterna crassissima* Blanch. is offered to supply the lack of an available treatise in English on the morphology of the beetles belonging to the family Scarabæidæ. The nearest approach to the subject is the famous historical French work of Straus-Durckheim "Considérations Générales sur L'Anatomie Comparée des Animaux Articulés" in which is included a description and many fine drawings of the anatomy of *Melolontha vulgaris*, Hanneton. This work appeared in 1828 and is a masterpiece of its kind. However, much of the anatomical nomenclature is now antiquated and the work itself hard to secure, consequently the present paper, dealing with a species of a closely allied genus of the same family, is here presented.

The nomenclature used by the writer is that which was deemed most useful. It follows no one system of the modern writers but is adopted from such writers as Snodgrass, Crampton, and others.

This species of the genus *Lachnosterna* was chosen for study because of its abundance in the vicinity of Manhattan, Kansas. Of the 23 species found in this vicinity, *L. crassissima* ranks first in relative numbers. A total of 86945 specimens of this genus have been collected by Mr. J. W.

[1] Contribution No. 54 from the Entomological Laboratory, Kansas State Agricultural College. This paper embodies the results of some of the investigations undertaken by the writer in the prosecution of project No. 100 of the Kansas Agricultural Experiment Station. The writer wishes to acknowledge his indebtedness to Dr. P. S. Welch for many helpful criticisms during the course of preparation of this paper and to the American Microscopical Society for a grant from the Spencer-Tolles Fund to publish the accompanying drawings.

McColloch and the writer during the years 1916–1920, and of this number 30230 were *L. crassissima*. The specimens were preserved in 70% alcohol, boiled in potassium hydroxide when ready to use, and studied under the binocular.

General Considerations

Size.—The adults of this species are among the most broadly ovate of the genus *Lachnosterna*. They vary greatly in length, width, and maximum depth, and, on an average, the females are somewhat longer than the males, as well as much broader posteriorly. The greatest body depth varies in individuals of both sexes. Not only in size is this difference noticeable, but also in respect to the regions of the body in the two sexes. The males have their region of greatest depth through the thorax, which was found to average 7.1 mm., while the females are deepest through the posterior end of the abdomen where they average 7.6 mm. Some of the depth variation in the females is due, in part, to the degree of flexibility of the intersegmental grooves, especially when the abdomen is distended with eggs.

Because of the deflexity of both the labrum and pygidium, the maximum length of the males and females was arbitrarily measured from the emargination of the clypeus to the basal or proximal edge of the pygidium and not to the extreme ends as is the usual practice. The average length of 25 males, chosen at random, was 18.2 mm. and, for the same number of females, 18.7 mm. The maximum length of the males was 20 mm. and of the females 20.9 mm., while the minimum was 16.3 mm. for the males and 16.5 mm. for the females. Width measurements were made at seven different regions of the body to get a general notion of the width variation. These regions were chosen arbitrarily as follows:

1—At anterior margin of the eyes
2—At anterior margin of prothorax
3—At lateral angles of prothorax
4—At base of prothorax
5—At base of elytra
6—At bulge near middle of elytra (widest points)
7—At declivity of elytra near the distal end.

The table on the opposite page shows the average width, length, and depth measurements of 25 males and 25 females.

In individual specimens, the length measurement is highly variable, depending on the character of the specimens at hand. Alcoholic and living specimens are extensile because of the telescopic nature of the union of head and thorax and to a lesser degree the thorax and abdomen, thus causing differences in length. Dried specimens will, on the contrary, permit of more constant measurements.

Color.—The general mass color of this species is chestnut-brown or castaneous (dragon's blood plus a slight admixture of vermilion—Smith's

color chart, 1906, p. 154). However, some specimens vary to a dark brown, almost black, in which case a grayish iridescence is very apparent. Dorsally, the head, thorax, and elytra are shining and in certain lights a gray color exists. This grayish tinge is a structural color caused by fine striæ on the elytra. These striæ are also present on the thorax but the iridescence is not so pronounced. The eyes are black and prominent. Ventrally the ground color is castaneous. The thorax is covered with dull, yellow hairs, 1.5–2 mm. long, somewhat sparsely scattered on the prothorax, but several times denser on the meso- and metathorax. The abdomen frequently has a grayish super-color imparted by an adhering exudation which can be scraped off. The legs and antennæ are lighter brown, almost ferruginous in color.

TABLE I.—AVERAGE SIZE OF VARIOUS BODY REGIONS

Sex	Average Length	Width							Greatest Depth
		Head	Prothorax			Abdomen			
		At Eyes	At Anterior Margin	At Lateral Angle	At Base	At Base	At Bulge	At Declivity	
	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.
Males...	18.2	4.7	5.2	7.2	7.8	8.4	9.8	7.6	7.1 At thorax
Females..	18.7	4.3	5.1	7.8	8.1	8.6	10.3	8.2	8.2 At abdomen

SEXUAL DIMORPHISM

Male.—The club of the antennæ (Plate I, fig. 4) is about equal in length to the scape (s.) and funicle (fu.) combined. At the middle of the ventral surface the abdomen is longitudinally depressed and the penultimate ventral segment bears a faint transverse carina near its distal end. Frequently, this carina is nothing more than a slightly wrinkled convexity. Immediately behind this carina the last, or ultimate, ventral segment has a deep, rounded fovea whose distal margin is obtusely and angulately emarginate. On the posterior tibia, the inner spur is about one-half the length of the outer and somewhat more slender spur. The pygidium is not gibbous and is more noticeably truncate at the distal end than is this structure in the female. The hind tarsi are longer than those of the female.

Female.—The club of the antennæ (Plate I, Fig. 5) is about as long as the funicle. The ultimate ventral abdominal segment is somewhat broadly and rather deeply emarginate distally, and the fovea is absent. The pygidium is gibbous, smooth, and shining at the apex of the gibbosity and more

exposed than in the male. Ventrally, the abdomen is more broadly rounded and shining than that of the male and the longitudinal depression is lacking. The inner spur of the hind tibia is about equal in length to the outer and about as wide as the same spur in the males, while the hind tarsi are shorter than in the male. The tooth of the tarsal claws is somewhat larger than this tooth in the male.

Contrast of Body Surfaces

Dorsal Surface.—The dorsum of the head, thorax, and abdomen, by a casual examination, appears smooth and shining, but closer scrutiny reveals minute punctures over the entire surface. These punctures are more numerous on the front than on any other part of the body. Here they are closely placed, and often confluent. The clypeus is less densely punctured and the punctures are about equal in density to those on the thorax. A setigerous canthus is found on the eye. On the thorax a faint, smooth, median, longitudinal line is formed by the absence of punctures. Laterally, the punctures are less dense. The lateral margin is serrate and hirsute, and at the base transverse channels extend mesad, failing to reach the median line. The scutellum is large, somewhat heart-shaped, irregularly and less densely punctured. The elytra are likewise irregularly punctured, and five indistinct costæ occur on each elytron.

Ventral Surface.—Ventrally, the head is dark-brown, sparsely hairy and, in part, concealed under the anterior ventral margin of the thorax. The thorax is thickly covered with pale, yellow hairs about 1.5 mm. long, and the legs are sparsely covered with shorter hairs. The punctures of the ventral abdominal surface are, on the whole, smaller than the dorsal ones. Each bears a short, recumbent hair and is more widely separated. There are about the same number of punctures per square millimeter as on the upper surface where they are larger but more closely placed.

Contour

The transverse contour of the body exhibits four distinct geometrical figures in three principal regions of the body. Through the head a narrow, elongated oval is apparent, through the thorax a broad oval, while the abdomen shows a somewhat different contour for each sex. In the female, it is broadly oval, almost circular, and in the male much the same, except for the ventral surface where the oval is somewhat flattened, due to the fovea on the lower surface of the abdomen.

Body Divisions

The three general regions of the body are to be recognized by definite sutures which separate them. The head is the smallest division, being less than one-half the width of the thorax. It is greatly depressed and deflected. From above only the clypeus, front and eyes can be seen, as

the labrum is deflected on the under side of the head. The head is telescoped within the thorax and connected to it by a sclerite-bearing cervicum or the so-called microthorax.

The thorax is next in size to the head, somewhat oblong, being nearly twice as wide as long. The sides are nearly parallel at the base, but converge anteriorly. The oval contour of the three segments of the thorax is nearly similar in each with perhaps a more flattened aspect of the prothorax. Dorsally, the meso- and metathorax each bear a single pair of wings, those of the mesothorax being modified to form the elytra which cover the metathoracic wings. Each segment of the thorax bears a pair of jointed legs.

The abdomen, which is larger than the other two divisions combined, is completely covered by the elytra, except at the pygidium. The pygidium is more exposed from above in the female than in the male. On the lower surface are found only six abdominal segments, while eight are apparent on the upper surface after removing the elytra. This condition is apparently constant in both sexes. The sexual differences of the abdomen have already been described. The abdomen is telescoped into the thorax on the lower side, and united to the metacoxæ. Dorsally, the connection is made by a membrane to the postscutellum, and the parts do not overlap. The intersegmental grooves on the dorsum are comparatively wide and the fusion of the segments is loose and flexible, while ventrally, the segments are closely fused, forming narrow, curved grooves. Seven pairs of spiracles are to be found on the abdomen, only one of which can be seen below the elytra. The others are mostly in the ridges formed in the pleura.

The Structure of the Head

Front and Vertex.—The size and general contour of the head (Plate I, figs. 1–2–3) have already been noted. It is partly withdrawn into the prothorax and the mouth parts are wholly on the inferior surface with but a small part of the labrum visible from above. The front (Plate I, fig. 1, fr.) is a large somewhat rectangular area lying between the eyes, and limited anteriorly by the suture separating it from the clypeus (cly.). Its outer angles are extended to form the canthus of the eye (cn.). No epicranial suture is present and the region is not sharply separated from the vertex. The front is closely and strongly punctate. Near the vertex, at the point where the prothorax overlaps the head, the punctures disappear rather abruptly, except near the eyes, leaving a strong line of demarkation between the punctured area and the smoother region of the vertex and occiput. A few scattered punctures are to be found in these regions. Each puncture bears a recumbent hair which is inclined anteriorly. The vertex (v.) merely consists of the upper region of the head having no definite

boundaries but lying between the front and the occiput; the occiput being the posterior region of the head lying above the opening of the occipital foramen. No ocelli are present.

The Clypeus and Canthus.—The clypeus (Plate I, fig. 1, cly.) is situated on the anterior margin of the front, and the suture separating them, which is strongly sinuate, is known as the clypeo-frontal suture. The clypeus is somewhat rectangular, being twice as wide as long. Numerous punctures are present but they are not as dense as on the front. The anterior margin is slightly emarginate and a strong upturning gives to the whole sclerite a deeply concave appearance. The postero-lateral corners of the sclerite are bordered by the eyes and at this point a chitinous process protrudes upon and partly divides the eyes. This process, known as the canthus (Plate I, figs. 1 and 2, cn.), appears somewhat as an extension of the clypeus, but in reality is a continuation of the anterior corners of the front lying partly under the clypeus. Hairs are scattered over the surface of the canthus.

The Labrum and Epipharynx.—The labrum (Plates I and II, fig. 1, labr.) is attached to the anterior border of the clypeus, being greatly deflected and nearly hidden by the clypeus. Dorsally it is somewhat semicircular in outline and is depressed to form a deep fovea near the center. It is covered with long thinly placed hairs. On its inner surface are two convergent rows of mesading point hairs (Plate II, fig. 1).

The epipharynx (Plate II, fig. 1, epi.) is greatly reduced in this species. It has almost disappeared because of the extension of the labrum over most of its entire surface. A somewhat triangular elevated clump of hairs, or spines, is the most conspicuous remnant of the epipharynx.

The Eyes.—The eyes (Plate I, figs. 1–2–3, e.) are the most prominent part of the head. They are large, somewhat oval bodies on the dorsal, lateral and ventral regions of the head. They are nearly divided dorsally by the canthus.

The facets (Plate I, fig. 6, fac.) which are about .021 mm. in diameter, average about 380 to the square millimeter. In shape they are somewhat regularly hexagonal and each hexagon is the cornea of a completely distinct eye.

Genæ and Gula.—The lateral parts of the epicranium form the genæ (Plate I, figs. 2 and 3, g.) whose ventral limits are determined by the sutures separating the genæ from the large head sclerite—the gula (Plate I, figs. 2 and 3, gu.). The gula occupies about one-third of the ventral surface of the head. It is somewhat quadrate in outline, being slightly wider anteriorly where it is separated from the submentum by a transverse suture. The lateral margins are limited by the gular sutures and posteriorly by the cervical membrane.

The Occipital Foramen and Tentorium.—The occipital foramen, or foramen magnum, is the large opening in the head, opposed to a like opening in the thorax.

Through the occipital foramen can be seen, within the head, a chitinous structure, the tentorium (Plate V, figs. 1–2–3, tent.). A large arch-like structure represents the body of the tentorium, while a pair of small, short, posterior arms (post. a.) are present. The anterior arms (ant. a.) are broad structures extending cephalad from the body of the tentorium, and the dorsal arms (dor. a.) are represented by a pair of short pointed processes extending cephalad into the head cavity.

The Cervicum and its Sclerites.—The membranous area between the head and thorax is known as the cervicum. It contains six cervical sclerites. No attempt to homologize these sclerites has been made. The drawing (Plate V, fig. 4) shows the left side of the cervicum with the dorsal edge to the right and the anterior edge toward the top. It will be seen that near the dorso lateral margin is a small hair-bearing sclerite and near the ventro lateral margin are two sclerites, a large anterior one which overlaps a small posterior one.

The Head Appendages

The Antennæ.—The antennæ (Plate I, figs. 4 and 5) have been previously mentioned under the discussion of sexual differences. The male (Plate I, fig. 4) has a much larger club than the female (Plate I, fig. 5). Normally both sexes have 10 segments, three of which go to make up the lamellate club (cl.). The others form the funicle (fu.) and scape (sc.).

The Mandibles.—The mandibles (Plate III, figs. 1 and 6) are large complicated structures bearing on their inner surface a large, oval, grinding, or molar surface (mo.). Extending over the molar surface are a number of transverse ridges which are used in the process of grinding food. The anterior end of the mandible is thought to be the homologue of the galea of the maxilla. It is modified into a sharp cutting edge with two blunt teeth (gal.). Near the anterior end of the molar surface is a membrane (memb.) bearing various shaped spines and setæ which are shown in Plate III, figure 5. Immediately caudad of the molar surface is still another membrane (memb.) bearing short broad stiff spines. These are shown enlarged in Plate III, figure 3. A transverse section through the molar at the junction of the ridges (Plate III, fig. 4) shows a flat surface with small ridges extended ectad over half the surface.

Two chitinous apodemes (Plate III, fig. 2) are attached to muscles controlling the movement of the mandible.

The Maxillæ.—Each maxilla (Plate II, fig. 3) is divided into five principal regions: the cardo, stipes, palpifer, galea and lacinia. There seem to be no sutures delimiting a subgalea or dividing the galea into two lobes. The cardo (cd.) is rather short and broadly club shaped, being constricted

somewhat posteriorly. Across the center is an abrupt change in contour, making the anterior region of the cardo much thicker dorso ventrally. This change of level is represented in the drawing by the transverse dotted line. The stipes (st.) is the large median triangular sclerite, alongside of which is the long narrow palpifer (max. pf.) bearing a four-jointed palpus (p.). On the margin of the stipes opposite the palpifer is a large somewhat triangular area with one corner elongated to form a large spine-bearing lobe. This is the lacinia (lac.). On the ectal margin of the lacinia is a large five-toothed heavily chitinized structure, the galea (gal.).

The Labium and Hypopharynx.—The labium (Plate II, fig. 2) is separated from the gula by a transverse suture which extends across the ventral surface of the head in the region where the cardo of the mandible is articulated. Following Kadic's (1902, pp. 207–228) interpretation of the labium of Coleoptera, we find the following regions:

The submentum is divided transversely into two regions, the anterior plate (Ap. Sm.) and the posterior plate (pp. sm.). The posterior plate is attached to the gula and is much wider at the postero lateral margins, somewhat constricted at the middle and slightly broader anteriorly. The anterior plate is more nearly quadrate, broader than long, and with the lateral edges rounding out to form a bulge near the middle of the sclerite. The mentum is separated from the anterior plate of the submentum by a transverse suture which has a distinct emargination near its center. Similarly, it is somewhat broadly quadrate and bears a few mesad pointing hairs. The anterior margin is strongly biemarginate.

The glossa and paraglossæ (Plate IV, fig. 1) are not evident on the ventral surface but are bent within the buccal cavity. The glossa (gl.) is a single median sclerite, while the paraglossæ (plg.) are found on either side of it. They bear the three-jointed labial palpi. These structures are not easily located without having well cleared specimens. Near the base of the palpus on the inner surface is a diagonal suture limiting an area termed the squama palpigera (sq. pl.).

The hypopharynx (Plate IV, fig. 1–3 hyp.) is a V-shaped spiny structure lying on a clump of spines or strong hairs, principally on the inner surface of the anterior plate of the submentum. Caudad and dorsad to the hypopharynx are two long, narrow, chitinous structures known as the fulcrum hypopharyngeum (ful. hyp.). At the dorso-posterior end are two small transverse sclerites constricted somewhat near their middle. These are the pharyngeal sclerites (phy. scl.). To these structures are attached the anterior margins of the pharynx (phar.) and just posterior to the hypopharyngeum, on each side, is a row of backward pointing hairs. Anteriorly, the two arms of the fulcrum hypopharyngeum unite under the pharynx to form a sort of strengthening apparatus for the spiny structure underlying the hypopharynx which extends forward to form three arms.

These are shown on their ventral aspect in Plate IV, figure 2. A lateral view showing the relation of these parts to the pharynx (phar.) is shown in Plate III, figure 3.

The Prothorax

Protergum.—(Plate VI, fig. 1). The tergum of the prothorax or pronotum is convex, nearly twice as broad as long, with the sides somewhat narrowing from the base to the apical margin and constricting rather suddenly anteriorly. The lateral margins are distinctly crenate and ciliate, but not so represented in the drawing. A deep emargination occurs on the anterior margin which overlaps the head, extending to the middle of the eyes. The posterior margin is broadly angulate and overlaps, as far as the elytra, the mesothorax to which it is connected by a membrane. Close, though not very dense, punctures cover the surface, and a smooth median caudo-cephalad line is faintly evident. On each side near the posterior margin is an incipient channel extending from the postero-lateral angle to within a short distance of the middle.

Pleura.—At the lateral margins, the tergum is not inflexed to form the so-called prothoracic epipleuræ which are strongly evident in some Coleoptera (e.g. *Pterostichus californicus* Dej.). The prosternum (Plate VI, fig. 2, pro. ster.) and the pleural sclerites compose the ventral aspect of the prothorax. The episternum (Plate VI, fig. 2, eps.) and the epimeron (epm.) have no line of separation. Anteriorly, the episternum elongates mesally to fuse with the sternum whose anterior margin turns inwardly to form a phragma. Likewise, the epimeron extends mesally, tapering towards its extremity. Thus the two extensions form the anterior and posterior margins of the coxal cavities (cc.). The junction of the epimeron with the posterior region of the sternum creates in other Coleoptera the closed coxal cavities. These are partly open. This species has no suture, as in *Melolontha vulgaris*, separating the sternum from the episternum.

Prosternum.—The prothoracic sternum (Plate VI, fig. 2, pro. ster.) occupies the inferior, median region of the prothorax. It is quite irregular in shape and has, as mentioned before, no distinct line of demarkation separating it from the episternum. Externally, a noticeable feature is a caudad-projecting tongue between the cavities of the coxæ. At the anterior end of this tongue an irregular, circular ridge causes the formation of a somewhat rounded depression of the sternum. This tongue-like projection after attaining the posterior margins of the coxæ is expanded at right angles and extends laterally to meet the epimeron of each side. Internally, after the removal of the coxæ, the sternum will be found to have enlarged into a somewhat rectangular piece with rounded postero-lateral corners. It tends to form a concavity in which a part of each coxa rests. On the sides of the anterior edge of the internal sternum are two prolonged

entosternal apophyses (Plate VI, fig 3, es. aph.) which extend dorso-laterally.

The first pair of spiracles located ventrally are suspended in the membrane which unites the prothorax to the mesothorax.

The Prothoracic Legs

The Trochantin.—(Plate VI, fig. 6, tn.). The trochantin is a small piece hidden within the interior of the prothorax, which, when viewed from its caudal aspect, presents a depressed or cup-like structure articulating with the anterior margin of the coxa. The latero-dorsal margin bears a small, somewhat sharpened corner that is loosely articulated with a small apodeme on the inner surface of the prothoracic episternum. The end of the coxa articulates with the lower end of this same apodeme.

The coxa.—(Plate VI, fig. 6, cx.). The coxa of the anterior leg is cylindrical in form, and slightly over three time as long as its greatest diameter. It lies transversely in the coxal cavity of the prosternum and extends laterally under the edges of the pleura, thereby concealing the articulation with the trochantin. On the inner surface is a large opening extending from near its lateral extremity to nearly half its length. The cephalic edge of this opening articulates with the trochantin and the caudal edge is connected by a membrane to the arms of the epimeron lying immediately behind. The opening is partly closed by an overlapping of its edges which serve as places of attachment for several muscles.

At the distal end the coxa likewise articulates with the sternum near the mid-ventral line, and is thus fixed at both ends so that it moves in a rotary manner on its axis. There is a second opening at the distal end which receives a prolongation of the trochanter and thus permits of articulation at this point.

The Trochanter.—(Plate VI, fig. 6, tr.). The trochanter is a small triangular piece lying between the coxa and femur, articulating with both the coxa and femur, being more firmly attached to the latter.

The Femur.—(Plate VI, fig. 6, f.). The femur is about as long as the coxa, is somewhat flattened and bears on its inner surface a groove-like depression in which the tibia may rest when folded back on the femur. A socket is located in its distal end which receives a condyle from the tibia, forming a ball and socket articulation between the femur and tibia.

The Tibia.—(Plate VI, fig. 6, t.). The tibia is remarkably adapted for burrowing in the soil. It is somewhat obliquely truncate at its apex, about equal in length to the femur, and is strongly compressed, especially at its anterior edge which bears the three tibial teeth. Of these the terminal tooth is very strong and about as long as the first tarsal joint, while the other two are broader and not so long. Near the femur the tibia is rather cylindrical and bears a terminal condyle for articulation with the

femur. On its external margin, opposite the three teeth, is a strong movable spine.

The Tarsus.—(Plate VI, fig. 6, tar.). The tarsus is composed of five segments. The first four are about of equal length, the fifth slightly longer. They are cylindrical, and become enlarged distally. The terminal segment bears a pair of large claws (t. cl.) each of which bears an intramedian (male) or median (female) tooth. The tooth is slightly larger in the female.

The Mesothorax

The Mesotergum.—(Plate VII, figs. 1, 2, 3). The mesothorax is the smallest of the three divisions of the thorax; it bears the second pair of legs and the first pair of wings modified to form the elytra (Plate VI, fig. 4). The dorsal or tergal region of the mesothoracic segment is occupied largely by the somewhat triangularly shaped scutellum adjoined to which are the points of attachment of the elytra (Plate VI, fig. 5). Three regions of the four which comprise the typical thoracic tergum (Snodgrass, 1909, p. 523) are to be distinguished in this species, namely, the praescutum, scutum and scutellum. The postscutellum, or pseudonotum of some writers is absent.

The Præscutum (Plate VII, figs. 1 and 2, praes. ph.) is composed of a small meso-cephalad projecting phragma between the prothorax and the metathorax. It is slightly concave and is reinforced at its cephalic margin by a somewhat heavier deposition of chitin, which forms a rod-like brace extending from one edge of the præscutum to the other (Plate VII, fig. 3). The scutellum (Plate VII, figs. 1, 2, 3, scl.) is a large somewhat triangularly shaped sclerite part of which is covered by the elytra leaving the posterior half exposed externally. Its anterior or covered portion is somewhat closely punctate and covered with recumbent hairs while the exposed part is sparsely punctate, devoid of hairs and rather shining. The lateral margins each bear, on their anterior halves, two mesad-projecting pieces which represent the divided regions of the scutum (Plate VII, fig. 2, sct.). These phragma-like pieces are nearly triangular in shape and each terminates in a pointed ventro-cephalad projecting process which rests on the underlying metathoracic præscutal phragma (Plate VII, fig. 2, prs. ph.), and articulates with its antero-lateral projecting corners. At the cephalic margin of the scutum is a small depression or cavity in which the third axillary of the elytra lies when in the state of rest. The caudal margins are extended backward and unite to form a semicircular reduplication on the inferior surface of the exposed portion of the scutellum (Plate VII, fig. 3, scl. red.). To this reduplication is attached a small membrane which connects the mesothoracic tergum to the lateral and caudal margins of the metathoracic præscutal phragma. The cephalic margin of the scutellum bears a membrane which connects the prothorax and mesothorax. The

cephalo-lateral angles of the scutellum articulate with a small, sharp process which projects ventrad and unites with the anterior margin of the mesoepisternum.

The Elytra.—(Plate VI, figs. 4 and 5). The elytra which cover the meso- and metathorax and the greater portion of the abdomen are large, somewhat rectangular wing covers extending caudad to the middle of the penultimate abdominal segment, leaving the pygidium exposed. Their lateral and posterior margins are somewhat abruptly declivitous. The upper surface bears faint traces of the nervures and at each humeral angle there is a slight protuberance. The elytra are inserted on the mesothorax between the scutellum and the mesopleura. The base of the wing covers is somewhat truncated and curves ventrad. Near the middle of the basal margins on each elytra is a strong, bifurcated apophysis (Plate VI, fig. 5), which articulates with the wing process of the mesothorax, there are three principal wing axillaries (Plate VI, fig. 5, 1 ax., 2 ax., 3 ax.) in the membrane which are very irregular in shape and impart a different appearance from every aspect in which they are viewed.

The interlocking mechanism of the elytra is similar to that described for *Lachnosterna fusca* by Breed and Ball (1908, p. 291) who found in Coleoptera four devices for fastening the elytra in place. These are described by these writers as follows:

1. By a co-adaptation of the elytra along the dorsal suture.
2. By means of a groove on the dorsal face of the metathorax into which the swollen inner edges of the elytra fit.
3. By slipping the anterior edges of the elytra under the scutellum and hooking them (a) on to the scutellum, or (b) on to the metathorax. Pressure derived from the retracted prothorax may aid in keeping these edges in position.
4. By hooking the anterior lateral edges of the elytra over ridges or into grooves on the lateral faces of the metathorax.

In *Lachnosterna*, the first three methods are used to interlock the elytra while the fourth is present but not functional.

The Mesopleura.—(Plate VII, figs. 4 and 5). The mesopleuron consists externally of two sclerites, the episternum (eps.) and the epimeron (epm.). The episternum is a subrectangular plate with a strongly rounded dorsal margin, which adjoins the alar membranes. The anterior and posterior margins are nearly parallel, the former serving as a place of attachment for the intersegmental membrane, and the latter bordering on the epimeron. The ventral margin is attached to the mesosternum (ms. ster.). The epimeron (epm.) is nearly trapezoidal in shape with the cephalic and caudal margins nearly parallel. The cephalic margin connects with the episternum, the caudal one joins the metathorax, the dorsal margin gives attachment to the alar membrane and the ventral margin tapers to meet and

connect with the coxa of the mesothoracic leg (cx.). Between the coxa and the episternum is a small, narrow sclerite not visible externally—the trochantin (Plate VII, fig. 4, tn.) which articulates by means of a small condyle with the coxa. This sclerite is not present in the corresponding region of the metathorax.

An internal view of either of the mesopleura (Plate VII, fig. 5) shows a strong entopleural structure arising along the suture separating the episternum and epimeron and forming a pleural ridge which tapers at its ventro-mesal angle into a pleural arm (pl. a.) extending into the body cavity and terminating in a cup-shaped disk which serves for the attachment of muscles. The caudal margin of the epimeron presents internally a strong reduplication which aids in concealing the spiracles of the second pair of respiratory organs. The spiracles are not visible externally but lie in the suture between the mesoepimeron and the metaepisternum. The dorsal margin of the episternum is modified into a strongly chitinized blunt process, constituting the wing process on which articulates the bifurcated apophysis of the elytron.

The Mesosternum —(Plate VII, figs. 6 and 7, ms. ster.). The mesosternum is a transverse quadrilateral plate whose anterior margin serves as a place of attachment for the intersegmental membrane lying between the pro- and mesothorax. Its lateral edges border on the ventral margin of the mesoepisternum, and the posterior margin presents a biemarginate appearance with a median, caudal projecting piece which extends between the coxæ. The whole external posterior margin is bordered by the mesocoxal cavities. With the coxa removed, it can be observed that the posterior margin is extended into a concave process in each cavity in which the coxa lies at rest. The extension joins the metasternum caudad of the coxæ.

The internal surface of the mesosternum (Plate VII, fig. 7, ms. ster.) shows a furcate process arising from the anterior portion of the concavities occurring in the coxal cavities. It consists of two anterio-dorsal pointing arms forming the so-called entosternum, or mesoentosternum, of the mesothorax. These arms are supported near their middle by a chitinous of the cephalic margin of the mesosternum.

The Metathorax

Metatergum—(Plate VIII, figs. 1 and 2). The four typical tergal regions are present in the metathorax. The præscutum (prs. ph.) is represented by three distinct pieces, a large semi-oval median prephragma or præscutal phragma separated from the scutum by a large membranous area and two lateral parts (praes.) which support the large præscutal phragma. The scutum (sct.) is composed of two large lateral halves separated by the notal groove (n. g.) containing the scutellum. The scutal halves are divided diagonally into an anterior and posterior region. The

anterior region bears laterally the anterior notal wing process (a. n. p.) and the posterior region (the so-called "*scapulaire posterieure*" of Straus Durckheim) carries laterally the posterior notal wing process (p. n p.) and the axillary cords (ax. c.). The diagonal line of demarkation causing the division of the scutal halves is the outer evidence of an internal ridge (Plate VIII, fig. 2, d. rd.) on the interior surface of the scutum. The metascutellum (Plate VIII, fig. 1, scl.) is a two-lobed piece at the posterior median angles of the scutum. It elongates cephalad to form a tongue-like process, which lies in the notal groove and is limited anteriorly and dorsally by the membrane separating it from the præscutal phragma and internally by the entodorsum or V-shaped ridge (ent. d.) on the internal aspect of the metatergum. The postscutellum (pss.) is a large, irregular piece lying immediately behind the scutellum and scutum. It bears the post-phragma (post. ph.), is inflexed mesad to furnish attachment for several muscles and also bears the membrane which connects the thorax with the abdomen. The lateral edges are inflexed caudad of the alar membranes and articulate with the epimera of the metathorax.

The Wings.—(Plate VII, fig. 9). The second or metathoracic pair of wings, which are membranous, are borne on the metathorax and are inserted between the metatergum and the metapleura in the alar membranes. In a state of rest the wings are transversely folded under the elytra and in flight extend nearly at right angles to the body. The wings are articulated to the body by four axillary sclerites (Plate VII, fig. 10, 1 ax., 2 ax., 3 ax., 4 ax.) similar to those described in *Melolontha* (Straus Durckheim p. 109), one of which (4ax) according to Snodgrass (1909, p. 545) is an accessory plate not corresponding to the fourth axillary in other forms. The first axillary lies laterad of the scutum and its anterior outer margin abuts the basal enlargement of the subcostal vein of the wing. Between the first axillary and the bases of the radius and medius lies the second axillary which is partly overlapped by the first. The third is larger than the second, and lies at the bases of the cubital and anal veins, while the fourth axillary is quite small and lies between the first and third axillaries.

The Metapleura.—(Plate VIII, figs. 3 and 4). The metapleuron is composed of two principal sclerites homologus to those of the mesopleuron —the metaepisternum and metaepimeron—each of which is subdivided into two regions. The lower division of the episternum or katepisternum (keps.) is an irregular semioval piece attached to the lateral margin of the metasternum. Its dorsal and posterior margins are connected to the lower edge of the epimeron. Dorso-anteriorly the episternum exhibits the second subdivision or anepisternum (aeps.). This is an irregular shaped piece bordering on the cephalic edge of the epimeron and to which is fused the lower part of the preparapterum (pptm.) which is likewise fused with

the base of the wing process (w. p.). Internally the preparapterum bears a large muscle disc or pronator disc (pn. d.).

The epimeron is divided into two parts, the katepimeron and the anepimeron. The latter (aepm.) lies immediately above the katepisternum and is elongated anteriorly to form the wing process (w. p.). The katepimeron (kepm.) is a quadrilateral piece lying caudad of the anepimeron. Just above the epimeron is the alar membrane in which is located the wing axillaries. The epimeron is connected posteriorly by an articulation with the postscutellum on its lateral edge. On its inner surface the suture between the episternum and epimeron is extended to form the pleural ridge which elongates into an adfurcal process (pl. a.) that rests on the lateral arm of the mesoentosternal furca. The ventral end of the pleural ridge extends to the coxa.

The Metasternum.—(Plate VII, figs. 6 and 7). The metasternum occupies the lower surface of the metathorax. It is much the same in shape as that of the mesothorax, but considerably larger and lies between the meso- and metacoxæ. On the internal surface of the sternum (Plate VII, fig. 7) is the large endosternum projecting dorsally (Plate VII, fig. 8). It consists of two laterally projecting arms which furnish support for the adfurcal processes of the entopleura and a large and somewhat pointed cephalad projecting arm. A manifestation of this structure is discernible on the outer surface of the metasternum in the form of a faint mid-ventral line.

The Metathoracic Legs

The metathoracic legs are different in structure, especially in the form of the tibiæ from the prothoracic legs which, as mentioned before, are remarkably adapted for burrowing. While this modification is not present in the hind tibiæ, broadly speaking the metathoracic legs are quite similar to those of the mesothorax which for this reason are not treated in this discussion. In the metathoracic legs a well marked sexual difference, to which reference has been made, is apparent in the distinctly longer tarsi of the male. The trochantin of the metathoracic legs is absent, although it is to be found both in the pro- and mesothoracic pairs.

The Coxa.—(Plate VIII, fig. 5, cx.). The coxa of the metathoracic leg is attached to the posterior margin of the ventral surface of the metathoracic segment, and likewise serves as a place of attachment for the intersegmental membrane lying between the thorax and abdomen. Externally it presents a flattened surface in the same plane as the metasternum and like the coxa of the prothoracic legs is more or less immobile, except in a semi-rotary manner. It lies at right angles to the longitudinal axis of the body and extends from the elytra at the lateral margin to the mid-ventral line. Internally it presents a hollow arrangement near the opening

of which is a chitinous ridge or infolding that permits the attachment of the flexor muscles.

The Trochanter.—(Plate VIII, fig. 5, tr.). The trochanter of the metathoracic leg is similar to that of the two other pairs of legs. It lies between the coxa and femur and is triangular in shape.

The Femur.—(Plate VIII, fig. 5, f.). The femur is slightly longer than the coxa. It is somewhat flattened with rounded edges, tapering toward the distal end where it articulates with the tibia.

The Tibia.—(Plate VIII, fig. 5, t.). The tibia of each meso- and metathoracic leg differs from that of the prothoracic leg in that there is no flattened modification for digging and burrowing as is present in the front leg. The proximal end articulates with the femur and the distal end with the tarsus, where it is slightly broadened and bears two sharp spurs varying in size in the two sexes. These have been described in the paragraph on Sexual Dimorphism.

The Tarsus.—(Plate VIII, fig. 5, tar.). The tarsus is similar to that of the other legs in having five segments. The terminal one has two sharp claws, each bearing a median tooth.

The Abdomen

The abdomen of *Lachnosterna* almost equals in volume the remaining portions of the body, and is directed in the horizontal plane. At its base it equals the thorax in size, to which it is attached throughout its complete circumference. Dorsally, it is connected by a membrane to the postscutellum and thereby conceals the postphragma. Ventrally, it is joined to the posterior edge of the internal opening in the metathoracic coxæ.

Concerning the number of evident (not actual) abdominal segments in this species, there are six ventrally and eight dorsally, while the actual number is perhaps eight ventrally and nine dorsally (Plate VIII, fig. 8). The ninth or terminal segment is reduced in size with only the ventral portion apparent externally, while the dorsal part is modified to form an infolding within the anal opening and is not visible from the exterior. Each segment, with the exception of the first, consists of two principal parts, a dorsal or tergal region, and a ventral or sternal area. The dorsal and ventral sclerites are united laterally by a membrane which permits dilation and contraction of the abdomen. Posteriorly, the membrane disappears, leaving no such separation between the terga and sterna of the terminal segments.

The Terga.—Nine terga are present. The elytra in the state of rest cover the first six terga which are only slightly chitinized. There is evidently not the necessity of heavier chitinization which characterizes the remaining or unprotected parts of the body. These terga are united by comparatively wide membranous areas which are larger laterally than

near the median region. The two remaining visible terga (seventh and eighth) are not protected by the elytra and are consequently densely chitinized as is also the ninth which has disappeared within the anal opening. The seventh and eighth are the widest tergites. They make up the pygidium and are more closely fused than the anterior terga.

The Sterna.—The first sternite has disappeared and only a rudiment of the second is present which is covered by the metathoracic coxæ. The remaining segments are more heavily chitinized than the second, are closely fused to each other, and permit of no movement as in the terga. The male sternites (Plate VIII, fig. 6) are somewhat flattened and the eighth sternite has a rounded fovea which is absent in the female (Plate VIII, fig. 7). These differences, due to sex, have been mentioned in the discussion of sexual dimorphism.

The Spiracles.—There are nine pairs of spiracles, two thoracic and seven abdominal. The first pair of prothoracic spiracles is located ventrally, and each spiracle is suspended in the membrane which unites the prothorax to the mesothorax. The second pair is not visible externally but is to be found in the suture between the mesoepimeron and the metaepisternum. The third pair, or first abdominal pair, lies dorsally in the membrane between the metathorax and the first abdominal segment. The next five pairs are found on the ridge formed between the tergites and sternites. These six pairs of abdominal spiracles are covered by the elytra. The last, or posterior pair, is exposed below the elytra on the seventh abdominal segment and lies in the suture between the sternum and tergum.

The Genitalia.—The genitalia properly speaking are perhaps more concerned with internal anatomy and should be discussed in such a treatise. However, they possess chitinous structures which are tegumentary in nature and will, therefore, be briefly discussed here, chiefly because of their importance as specific characters in taxonomy.

In the male (Plate IX, figs. 2 and 4) a heavily chitinized semicylindrical sheath or box, termed the telum (Plate IX, fig. 4, te.), surrounds the true membranous penis. The posterior end is shown in perspective in the drawings showing its relation to the claspers which surmount the telum and in this species are rather symmetrical. These claspers (Plate IX, fig. 2, cls.) are of much taxonomic importance.

Underlying the telum is a small Y-shaped chitinous structure (Plate IX, fig. 3). Posteriorly, the branching arms are bent dorsally and to them is attached the membrane which constitutes the anterior region of the cloaca. The membrane is also attached to the inner margin of the last ventral and abdominal segments. Anteriorly, this structure extends into the body as far as the sixth ventral abdominal segment.

The female genitalia (Plate IX, fig. 1) are shown in three views. The organ consists of a pair of broad inferior plates (inf. pl.) which surround a

smaller pair of superior plates (sup. pl.) somewhat cylindrical in shape and strongly divergent.

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List of Abbreviations

a.	anal opening	a.n.p.	anterior notal wing process
a.a.	anterior arm of endosternum	ap.sm.	anterior plate of submentum
1_a 2_a 3_a	first, second and third anal veins	ax.c	axillary cord
aepm.	anepimeron	c.	costa
aeps.	anepisternum	c.c.	coxal cavity
ant.	antenna	cd.	cardo
ant.a.	anterior arm of tentorium	cdy.	condyle

cl.	club
cls.	claspers of male
cly.	clypeus
cn.	canthus
cu_1 cu_2	first and second cubitus
cx.	coxa
cx.ph.	coxal phragma
d.rd.	diagonal ridge of scutum
dor.a.	dorsal arm of tentorium
e.	eye
ely.	elytra
ent.d.	entodorsum
ent.ster.	entosternum
epi.	epipharynx
epm.	epimeron
eps.	episternum
es.aph.	entosternal apophysis
f.	femur
fac.	facet
fc.	furca
fr.	front
fu.	funicle
ful.hyp.	fulcrum hypopharyngeum
g.	gena
gal.	galea
gl.	glossa
gu.	gula
hyp.	hypopharynx
nf. pl.	inferior plates
kepm.	katepimeron
keps.	katepisternum
la.	lateral arm of entosternum
labi.	labium
labr.	labrum
lac.	lacina
post.a.	posterior arm of tentorium
m.	mentum
md.	medius
m.d.	muscle disc.
mand.	mandible
max.	maxilla
max.pf.	maxillary palpifer
memb.	membrane
mo.	molar
ms. ster.	mesosternum
mt.ster.	metasternum
n.	notum
n.g.	notal groove
oc.for.	occipital foramen
p.	palpus
pgl.	paraglossa
phar.	pharynx
phy.scl.	phryngeal sclerite
pl.a.	pleural arm—entopleurum
pn.	pronotum
pn.d.	pronator disc.
p.n.p.	posterior notal wing process
p.n.r.	post notal ridge
post.a.	posterior arm of tentorium
post.ph.	postphragma
pp.sm.	posterior plate of submentum
pptm.	preparapterum
praes.	praescutum
praes.ph.	praescutal phragma-mesotergum
prs. ph.	praescutal phragma-metatergum
pro. ster.	prosternum
pss.	postscutellum
r.	ridge
rd.	radius
s.	scape
sc.	subcosta
scl.	scutellum
sct.	scutum
scl.red.	reduplication of scutellum
sp.	spiracle
sq. pl.	squama palpigera
st.	stipes
sup.pl.	superior plate
t.	tibia
tar.	tarsus
te.	telum
t.cl.	tarsal claw
tent.	tentorium
tn.	trochantin
tr.	trochanter
v.	vertex
w.p.	wing process
1–9	abdominal segments
1 ax.	first axillary
2 ax.	second axillary
3 ax.	third axillary
4 ax.	fourth axillary.

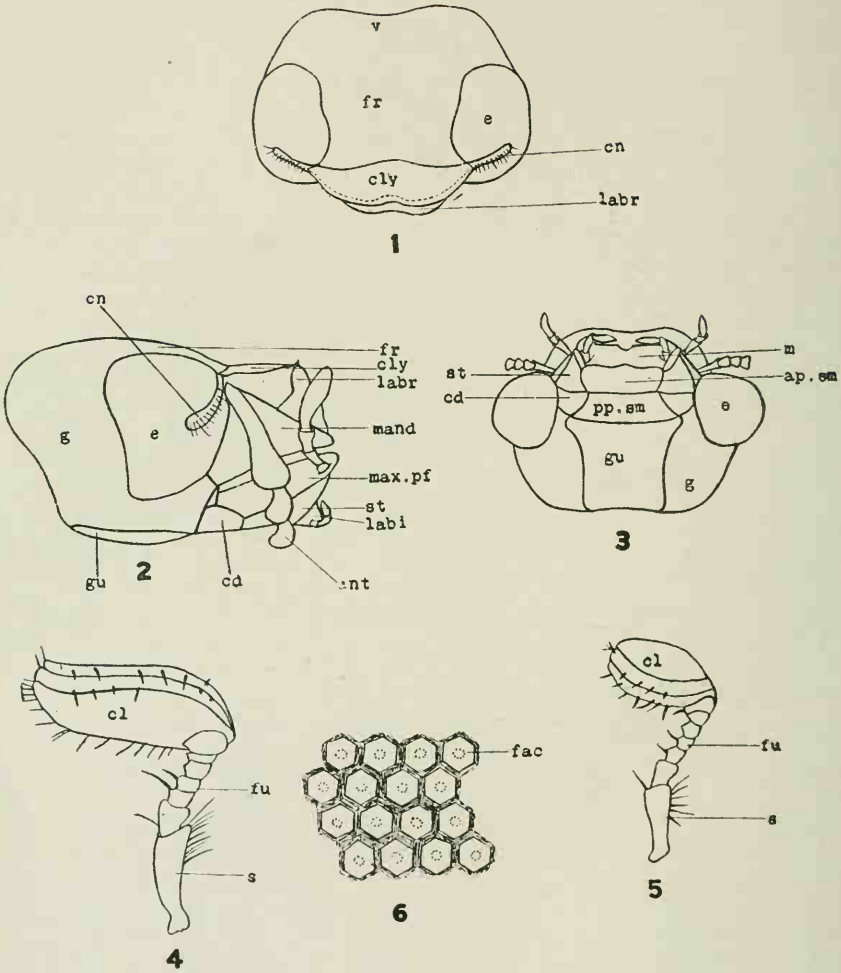
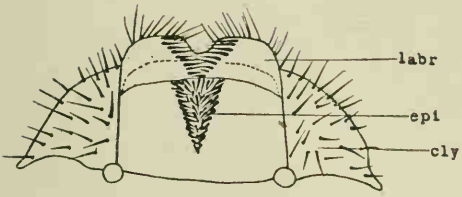


PLATE I

Fig. 1. Front view of head.
Fig. 2. Lateral view of head.
Fig. 3. Ventral view of head.
Fig. 4. Antenna of male.
Fig. 5. Antenna of female.
Fig. 6. Facets of the compound eye.



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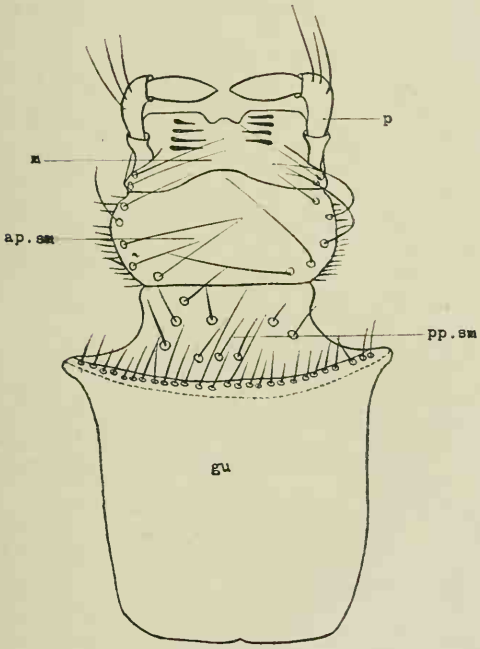

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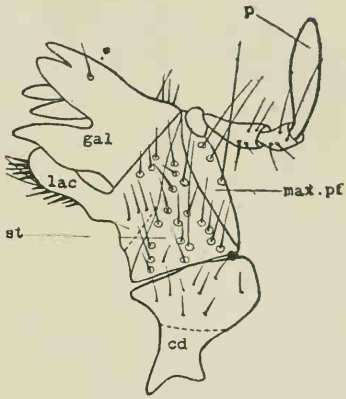

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PLATE II

Fig. 1. Epipharynx and internal aspect of clypeus and labrum.
Fig. 2. Gula and Labium.
Fig. 3. Left maxilla.

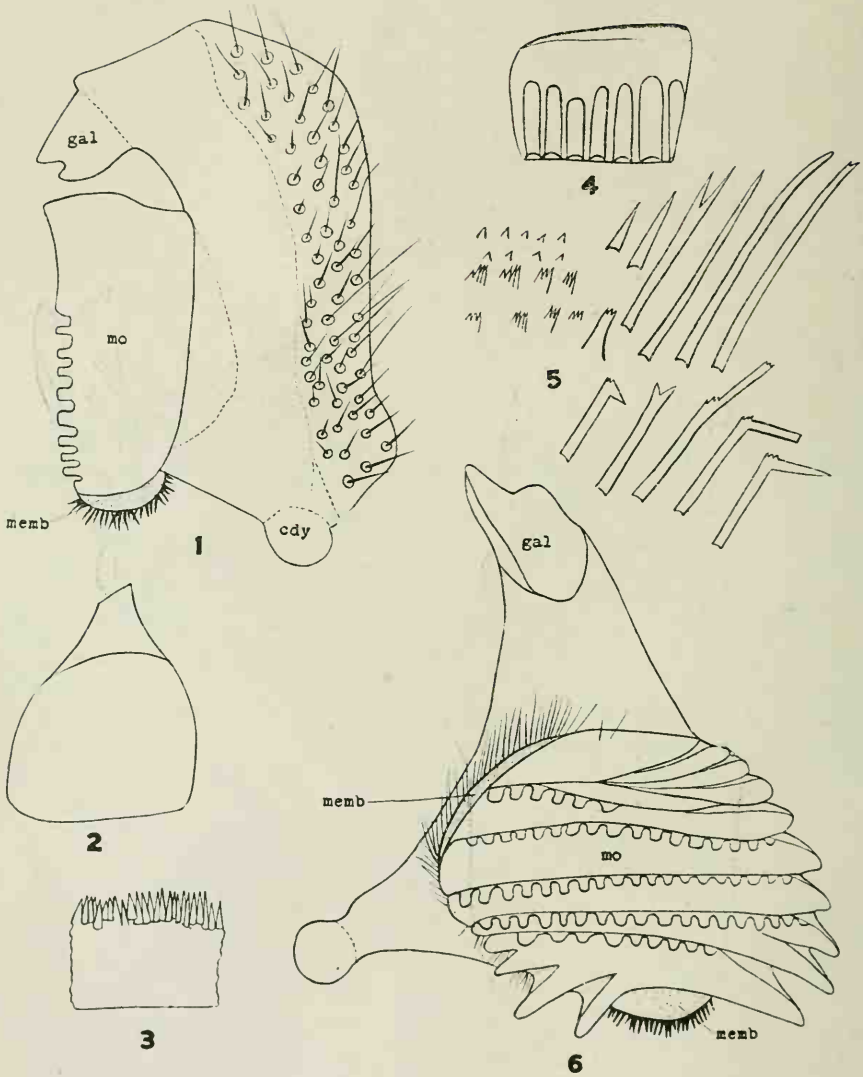

PLATE III

Fig. 1. Side view of right mandible.
Fig. 2. Apodeme of mandible.
Fig. 3. Membrane and hairs at base of mandible.
Fig. 4. Portion of cross section thru molar.
Fig. 5. Hairs and setæ from upper membrane of mandible.
Fig. 6. Inner surface of mandible.

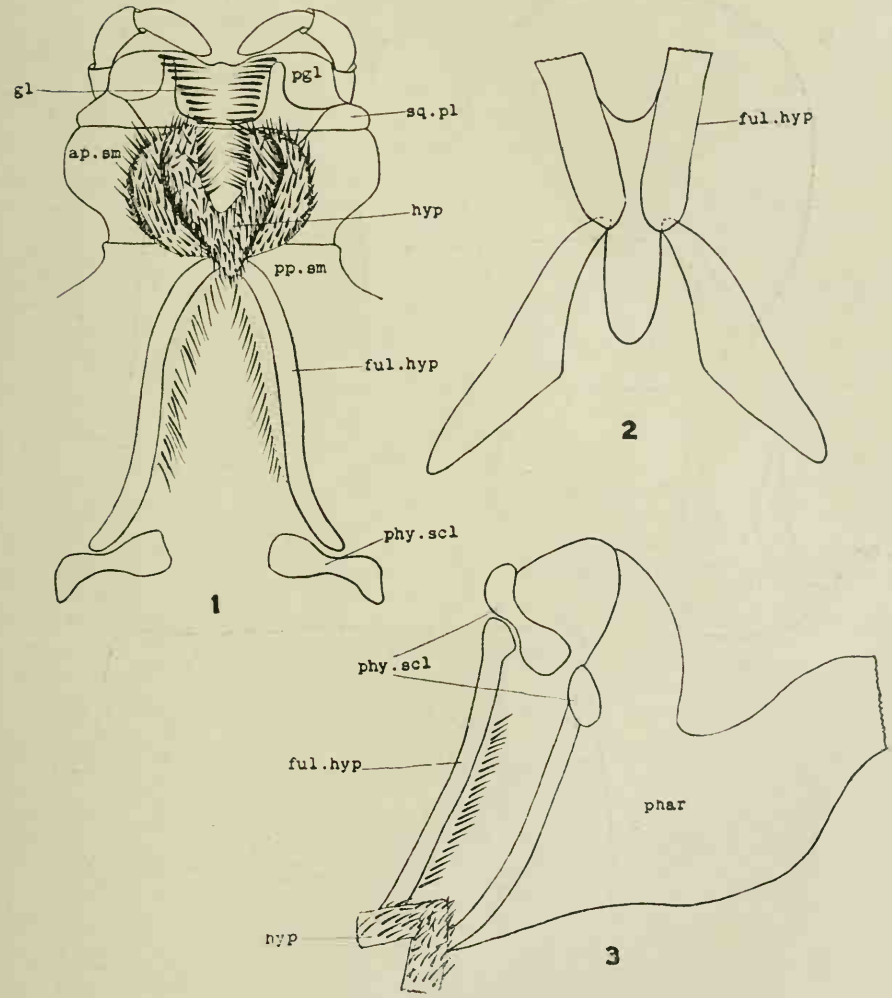

PLATE IV

Fig. 1. Labium from within showing hypopharynx and fulcrum hypopharyngeum.
Fig. 2. Junction of arms of fulcrum hypopharyngeum, ventral aspect.
Fig. 3. Lateral view showing relation of fulcrum hypopharyngeum to the pharynx.

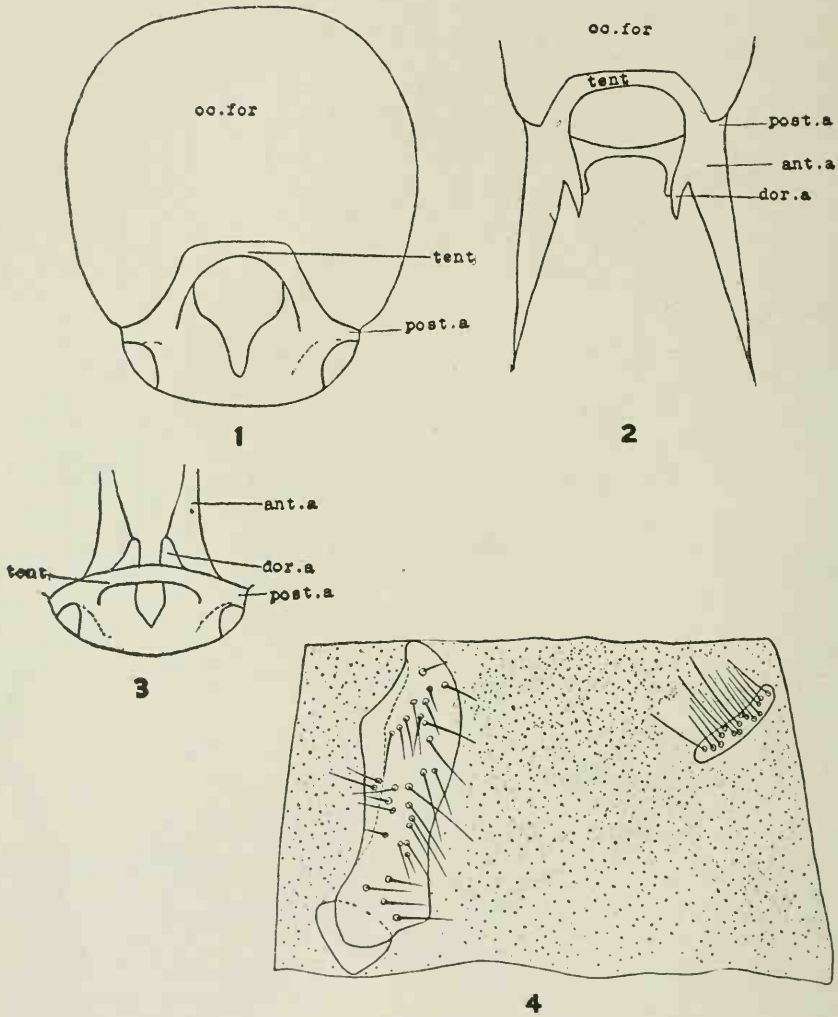

PLATE V

Fig. 1. Tentorium—looking through occipital foramen.
Fig. 2. Anterior view of tentorium.
Fig. 3. Dorsal view of tentorium.
Fig. 4. Left side of cervicum (dorsal margin to the right).

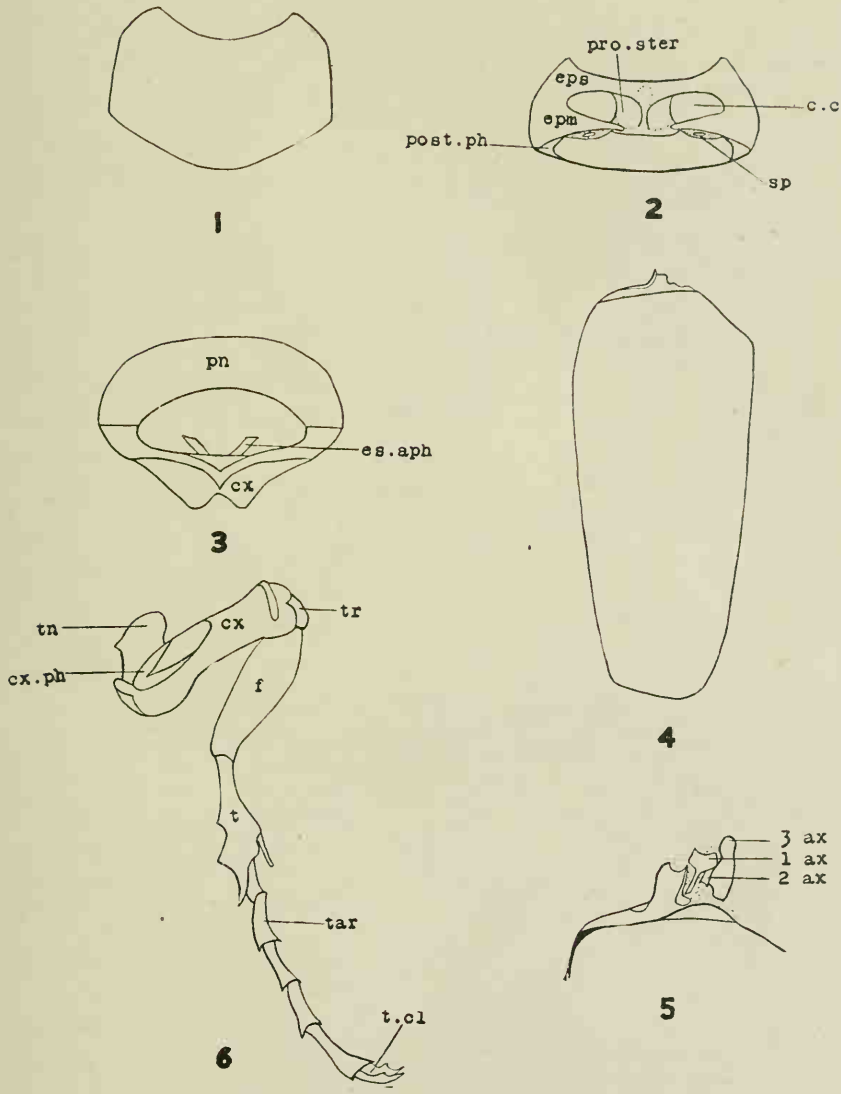

PLATE VI

Fig. 1. Dorsal view of prothorax.
Fig. 2. Ventral view of prothorax—coxæ removed.
Fig. 3. Anterior aspect of prothorax.
Fig. 4. Left elytron.
Fig. 5. Articulation of elytron.
Fig. 6. Right prothoracic leg.

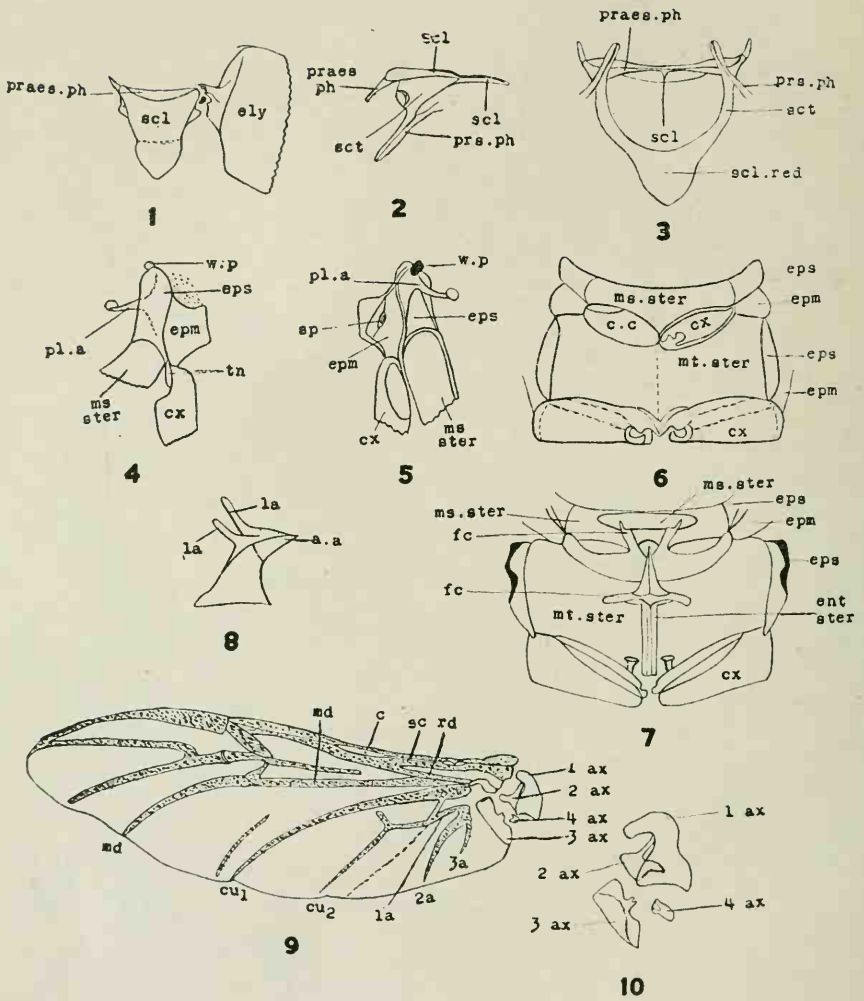

PLATE VII

Fig. 1. Dorsal view of mesothorax with portion of right elytron.
Fig. 2. Left lateral aspect of mesothoracic tergum.
Fig. 3. Internal aspect of mesothoracic tergum.
Fig. 4. Left mesopleuron, external aspect, coxa ex situ to show trochantin.
Fig. 5. Left mesopleuron, internal aspect.
Fig. 6. Meso—and metasterna, external aspect.
Fig. 7. Meso—and metasterna, internal aspect.
Fig. 8. Metathoracic endosternum.
Fig. 9. Metathoracic wing.
Fig. 10. Axillary sclerites of the metathoracic wing.

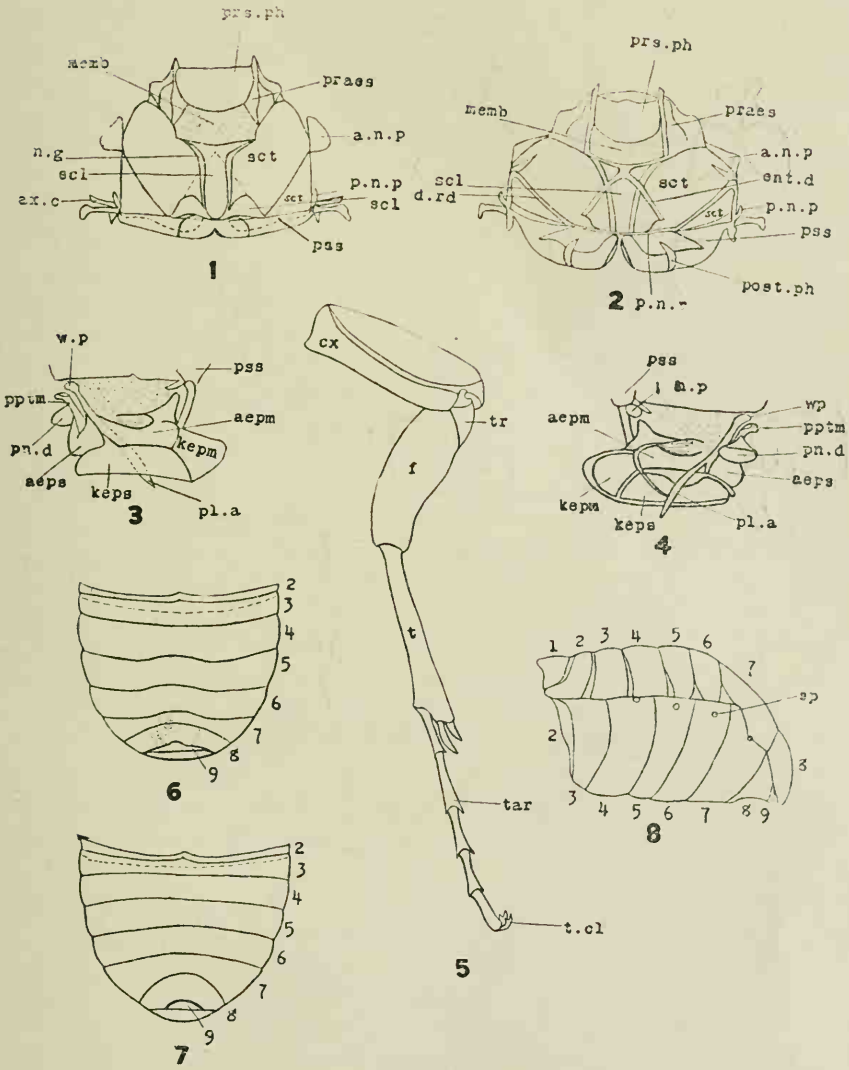

PLATE VIII

Fig. 1. Metatergum, external aspect.
Fig. 2. Metatergum, internal aspect.
Fig. 3. Left metapleuron, external aspect.
Fig. 4. Left metapleuron, internal aspect.
Fig. 5. Right metathoracic leg.
Fig. 6. Ventral aspect of abdomen, male.
Fig. 7. Ventral aspect of abdomen, female.
Fig. 8. Left lateral aspect of abdomen, male.

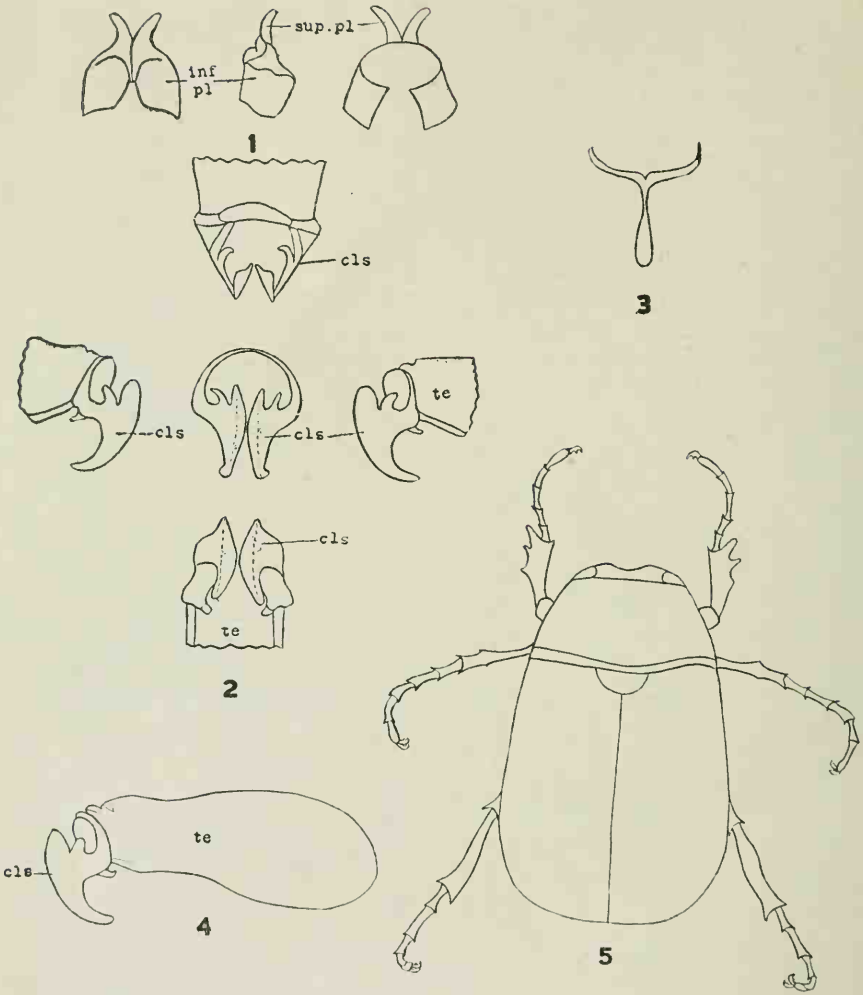

PLATE IX

Fig. 1. Female genitalia, dorsal, ventral and lateral aspects.
Fig. 2. Male genitalia, arranged perspectively.
Fig. 3. Y-shaped supporting structure of cloaca and telum.
Fig. 4. Male genital organ with telum in place.
Fig. 5. Dorsal aspect of *Lachnosterna crassissima*.

THE RESPIRATORY MECHANISM IN CERTAIN AQUATIC LEPIDOPTERA[1]

By
Paul S. Welch

Introduction

According to one of the current theories, insects arose from a terrestrial ancestry and the aquatic habit, wherever manifested, was secondarily acquired. The general application of this theory to all aquatic insects is sometimes questioned but there seems to be an almost universal agreement that many of the higher orders, including the Lepidoptera, are preëminently terrestrial in organization and that their aquatic representatives display an evolution superimposed upon a terrestrial background. These insects, invading water, had certain vital problems to solve, respiration being among the first, and the diverse but successful adaptations offer interesting material for study.

The writer has found the aquatic Lepidoptera favorable for the study of larval adaptations for the following reasons:

1. While the Lepidoptera constitute a large, well-defined order, the insignificant number of aquatic forms made it possible to examine most of the American species.

2. Although inconsequential numerically, they manifest adaptations to aquatic life as perfect and as diverse as many of the more conspicuous groups.

3. Heterogeneity in the methods of solving aquatic problems may appear even among species of the same genus.

4. The abundance of some species in certain environments has provided ample material for extensive observations and experiments.

5. This group of aquatic insects is practically unstudied from the morphological, physiological and ecological aspects.

Excluding the semi-aquatic forms, aquatic Lepidoptera fall into two general classes when considered from the point of view of larval respiration: (1) Those which are devoid of any special respiratory organs, and secure the requisite oxygen from the atmosphere by some physiological adaptation, from the dissolved supply in the water, or through a combination of both; and (2) those with special morphological devices in the form of gills. In the first class belong such forms as *Bellura melanopyga*, *Nymph-*

[1] Contribution from the University of Michigan Biological Station, and the Zoological Laboratory, University of Michigan.

ula icciusalis, and *Pyrausta penitalis*, while *Nymphula maculalis*, *N. obscuralis*, and *Cataclysta fulicalis* are typical representatives of the second.

Acknowledgments

The writer wishes to express his indebtedness to Professor S. A. Forbes, Professor J. G. Needham and Mr. J. T. Lloyd for certain materials specified on later pages. Use has been made of data accumulated by two of the writer's graduate students, Miss Ethelynn Hopkins and Mr. Jennings Hickman. During the summer of 1919, Miss Hopkins studied briefly the tracheation of certain instars in *Nymphula maculalis* and *Nymphula icciusalis*, using living material. Later, Mr. Hickman examined the gills of *Nymphula maculalis*, using serial sections of preserved specimens. The work of both of these students has been repeated and extended by the writer.

Material

The principal objects of the work on which this paper is based were to determine the structure and mode of function of the respiratory organs developed in this group; to study the degree of change from the original terrestrial organization of the larvae; to get some measure on the efficiency of special organs; to examine the various instars with respect to the respiratory problem; to test certain doubtful statements in the literature; and if possible to determine something as to the course of evolution in such a limited group.

In order to accomplish these ends it was necessary to study as many representatives of the two above mentioned classes of aquatic Lepidoptera as possible. Owing to the abundance of several species in the vicinity of the University of Michigan Biological Station, repeated observations and experiments have been made during the past four summers. Some of the necessary preliminary work on habits and life-history has been published already (Welch, 1914, 1915, 1916, 1919). The material used most extensively in the present work is as follows:

Non-gilled Larvae

Nymphula icciusalis Wlk.—This species occurs abundantly at Douglas Lake, Michigan, and several instars have been studied in both living and preserved form.

Nymphula obliteralis Wlk.—Preserved material of the larvae of this species was secured from the collections of the Illinois Natural History Survey through the courtesy of Professor S. A. Forbes. The life-history of this species was described by Hart (1895, pp. 176–180) and the specimens used were from Hart's collections and identified by him. Living material has not been available.

Nymphula sp.—Larvae of a species of *Nymphula* occasionally appeared at Douglas Lake on the leaves of the yellow water-lily but it has not been bred to the adult stage and its specific identity is not known.

Gilled Larvae

Nymphula maculalis Clem.—Living material in all stages of the life-history including larval instars is available in abundance at Douglas Lake, Michigan, during July and August. Observations were also made on living specimens at Lake Oneida, N. Y., during the summer of 1916. Liberal use was made of preserved material in connection with the morphological work on the spiracles and gills. The biology of this species (Welch, 1916) has already been described.

Nymphula obscuralis Grt.—Preserved larvae were received from the Illinois Natural History Survey. Hart (1985, pp. 167–173) reported on the life-history of this form and the specimens sent to the writer were from Hart's collections and identified by him. No living material was available.

Cataclysta fulicalis Clem.—Preserved larvae were sent to the author by Professor J. G. Needham and Mr. J. T. Lloyd, from collections made in the vicinity of Ithaca, New York. These gilled caterpillars have been described by Lloyd (1914, 1919) and are conspicuously different from the non-gilled *Cataclysta* forms in other parts of the world. Living material was not available.

The Tracheal System

Since the gilled caterpillars represent the greatest progress in the direction of special aquatic respiratory adaptation and since the gap between the gilled and non-gilled forms is without intergrades, it was of interest to compare the tracheal systems of species representing these two classes and to seek light on the following questions: (1) Do the non-gilled forms possess a system of tracheation built on the plan of terrestrial lepidopterous larvae? (2) Do the gilled forms have the same system of tracheation found in the non-gilled aquatic forms? or (3) Has the acquisition of gills been accompanied by noteworthy changes in the fundamental tracheation?

These questions are significant in connection with certain facts of general habits and life-history. There is little or no doubt at present that the non-gilled forms once included in the genus *Hydrocampa* and the gilled forms once included in the genus *Paraponyx* really comprise one genus, *Nymphula*, and they are usually so treated. In Douglas Lake and other similar lakes studied by the writer, it is commonplace to find the gilled caterpillars and non-gilled caterpillars thriving side by side in the same vegetation beds, subject to identical environmental factors. In *N. maculalis* the first instar is devoid of gills, these appearing only in the second instar, when forty gill-filaments come into existence. The gill-filaments

increase in number with each succeeding molt until the final larval instar has an equipment of over four hundred.

Non-gilled Larvae

A study was made, using living material, of the arrangement and distribution of the tracheae in the different larval instars of *N. icciusalis*, giving special attention to the early instars and the full-grown larva. Taking into account only such tracheae as appear under the higher powers of the binocular microscope and the medium powers of the compound microscope, and using only fresh preparations from which none of the air had been lost, it was possible to diagram the fundamental structure of the tracheal system and to make comparisons not only in the different instars but also with other species.

It has thus been found that the ground plan of arrangement and distribution of tracheae is essentially that which characterizes the terrestrial larvae. Deviations and minor variations appear but none of them seem to bear any significant relation to the acquired habits of the larvae. No important changes of any kind appear as later instars are reached.

Gilled Larvae

Detailed studies of tracheation in the larval instars have also been carried on with *N. maculalis* using fresh, living material. In the first instar the system is almost a duplicate of that in the first instar of *N. icciusalis*. In the second, the appearance of gills is accompanied by no consequential change, the gills being supplied by short direct branches from the main longitudinal tracheae. Also in the rapidly increasing gill complexity of the later instars there is no deviation which the writer can recognize as having any significance in relation to the aquatic habit or to the acquisition of gills.

The results of this part of the work seem to show that fundamentally these aquatic caterpillars have retained the original terrestrial form of body tracheation and that the gill tracheation is a system superimposed upon the one already present in the whole group. Gills have therefore been developed with a minimal change of the original tracheation. It would appear, if these conclusions are well taken, that the larval type represented by that of *N. icciusalis* is the older one phylogenetically and that the gilled caterpillar has a more recent origin.

Structure of the Gills

A detailed study of the structure of the gills in all of the species available was made by means of longitudinal and transverse sections of preserved material and by the examination of living material, whenever the latter could be secured. The relative transparency of the living material, especially when submerged in dilute glycerine, or some of the oils used for

clearing made it possible to study certain features more readily than in sections, particularly the distribution of tracheoles. Sections cut 6 microns thick and double stained in haemotoxylin and eosin gave satisfactory results. High magnification was often required for the examination of sections, particularly for the study of the histological features, and for the more critical and difficult features a 1.9 mm. oil immersion fluorite objective was used in connection with a monobinocular microscope.

The Gill-wall

A study of the gill-wall shows that it is essentially a continuation of the body-wall, having the same set of layers. In order to determine whether any differences in thickness appear, a number of measurements were made of the various layers of the gill and of adjacent portions of the body-wall, the averages being given in the following table. All of these measurements were made on specimens in the last larval instar. Measurements in this and succeeding tables are expressed in fractions of a millimeter. The terms *epidermis* and *dermis* are used instead of the *primary cuticula* and *secondary cuticula* of some authors.

	Epidermis	Dermis	Hypodermis	Total
N. maculalis				
Gill-wall.....	0.0004	0.0056	0.0017	0.0077
Body-wall.....	0.0004	0.0115	0.0027	0.0146
N. obscuralis				
Gill-wall.....	0.0012	0.0017	0.0009	0.0038
Body-wall.....	0.0005	0.0037	0.0010	0.0052
C. fulicalis				
Gill-wall.....	0.0037	0.0075	0.0025	0.0137
Body-wall.....	0.0037	0.0190	0.0033	0.0260

It thus appears that there is a distinct reduction of thickness in the walls of the gills as compared with the body-wall, this reduction occurring mainly in the dermis. The basement membrane is so delicate that it has been left out of account in all measurements of body-wall and gill-walls.

The Gill-cavity

The interior of each gill is merely a cavity (Pl. X, fig. 6) enclosed by the walls described above and containing certain structures to be discussed in another connection. This cavity extends continuously from base to tip and lacks completely, in the species examined, the alveolar type of tissue which appears within the gills of some insects. The size of this

cavity depends entirely upon the dimensions of the gill as a whole and has a direct connection with the haemocoele, in fact, it is a continuation of the haemocoele. In the Nymphula group, the gills are relatively large, have a spacious gill-cavity, and the passage from the haemocoele to the gill-cavity is broad, while in *Cataclysta fulicalis* the gill as a whole is small and slender, the wall thick, the gill-cavity much reduced, and the passage from the haemocoele to the gill-cavity often smaller in diameter than that of the gill-cavity. For example, in one series of eighteen measurements the average diameter of the gill-cavity was 0.0054 mm. while the average diameter of the opening into the haemocoele was 0.0039 mm.

Contents of the Gill-cavity

Tracheae

Nymphula maculalis.—As previously mentioned, the gills in this species are supplied with tracheal branches arising directly from the adjacent main longitudinal tracheae. Since the gills become branched in later instars, the supplying tracheae branch correspondingly. Each filament, therefore, has one main trachea, axial in position, which extends from the base almost to the tip, gradually decreasing in diameter distad. A very few instances of two tracheal branches entering a gill-filament were observed, both extending well towards the tip of the filament and both giving rise to tracheoles.

The origin and distribution of the tracheoles were best studied in living material, although certain data were confirmed by means of serial sections. At frequent but irregular intervals (Pl. XI, figs. 9, 10, 11) along the supplying trachea short tracheoles arise singly, extending ectad to the inner surface of the gill-wall and giving off numerous fine branches, all of which break up into very minute tracheoles and have a rather definite arrangement as follows. These tiny tubes all extend longitudinally, proximad and distad, very near or in contact with the ental surface of the gill-wall, and approximately parallel to each other, so that the periphery of the gill-cavity is bounded by a thin zone composed of countless, minute, parallel tracheoles. The terminal tracheoles of each individual tuft intermingle with those of the adjacent tufts but also in an approximately parallel fashion. All attempts to determine the character of the terminations of these tracheole endings, using the best preparations and the highest magnifications, have thus far been futile. In living material and in whole mounts they appear to unite with the basement membrane and serial sections confirm this conclusion, but nothing further can be said as to the exact relation to the gill-wall. No tracheoles were found lying free in the gill-cavity. The profusion of these tracheoles, intimately related to the entire inner surface of the gill-wall, points definitely to the principal function of these body projections.

Nymphula obscuralis.—Since it has not been possible to study living material of this species, the tracheation of the gills cannot be so definitely described. However, serial sections show a type of structure closely resembling that of *N. maculalis*. It is probable that both species have systems which are very similar.

Cataclysta fulicalis.—Serial sections including all parts of the body of the larva show no tracheation (Pl. X, fig. 5) of the gills. No branches of the body tracheal system approach the bases of these organs and in no sense are they to be regarded as *tracheal* gills.

Body Fluids

In living specimens of *N. maculalis*, it is easy to observe the movement of fluid, not only within but also into and out of the gill-cavity, thus giving added proof of the continuity of this cavity with the haemocoele. Blood corpuscles can be detected in this fluid. Sections confirm the observations on living specimens, showing that the gill-cavities invariably contain haemocoele fluids. Preserved material of *N. obscuralis* yielded similar results.

The small attenuated gill-cavities of *Cataclysta fulicalis* contain only the remains of fluid originating from the haemocoele.

Discussion

It thus appears, from the point of view of structure alone, that two distinct gill types occur in aquatic larvae of Lepidoptera: (1) combination tracheal-blood gills, and (2) blood gills.

As already pointed out, the profusion of tracheoles in each gill-filament in N. maculalis indicates the respiratory nature of these organs. With its equipment of over four hundred gill-filaments the mature larva apparently has more than ample provision for respiration, especially since these larvae live in surface water rich in dissolved oxygen, and often in vegetation beds which contribute additional oxygen. This gill equipment is also striking in view of the fact that certain non-gilled *Nymphula* larvae thrive in identically the same external conditions. *Nymphula obscuralis*, according to Hart (1895, p. 170), has an average of four hundred and eighty-four gill-filaments. In *Cataclysta fulicalis*, the number is smaller, the full-grown larva having about one hundred and twenty unbranched gills.

Both blood gills and tracheal-blood gills are known to occur in limited numbers in other orders of insects. There is no special difficulty in understanding the mode of functioning of the ordinary tracheal gill, but in the combination described above and in the blood gill of the *Cataclysta* type, certain problems arise, first of which is the nature of the relation, if any, of the blood (the body fluid which circulates in the gill-cavities) to the transportation of oxygen. In the blood gills of certain chironomids, the

blood contains haemoglobin and with this carrier present the gills have definite significance. In the larvae of *N. maculalis* and in certain other insects having similar gills, some carrier other than haemoglobin seems necessary to enable these structures to function as gills. The actual discovery of invisible carriers has not yet occurred. Muttkowski (1920, 1921a, 1921b, 1921c) suggests that possibly haemocyanin may constitute such a carrier. Rose and Bodansky (1920) demonstrated the presence of copper in a number of marine organisms and Muttkowski (1921a) found it in a large number of animals representing six phyla. The last named investigator holds that "Copper is found in insect blood in quantities comparable to that of crayfish blood. Its rôle is therefore interpreted as being identical,—namely that it serves as the nucleus of a respiratory protein,—hemocyanin. Insects, therefore, have two sources of oxygen,—atmospheric air led directly to the tissues by way of the tracheae, and fixed oxygen arried by the respiratory protein of the blood." Possibly this is a hint in the right direction and invisible oxygen carriers in insect blood may soon be identified.

Since the circumstances seem to demand the presence of some oxygen carrier, the question arises concerning the mode of functioning of the combination gill. Does such a gill have two separate and distinct methods of securing and distributing oxygen? The position and distribution of the tracheoles are such that there seems to be no ground for assuming any relation to the blood as an intermediary between them and incoming oxygen. Perhaps the tracheal system might function as completely if the gill-cavity were filled with alveolar tissue instead of blood. On structural grounds alone, it appears possible that two distinct methods could exist side by side. It might be suggested that in *N. maculalis* and *N. obscuralis* the gills are really tracheal gills and that the presence of blood in the gill cavity is entirely incidental, but such a suggestion loses weight when *C. fulicalis* is considered since its gills, if they function at all, must do so through the intermediation of the blood. It has not been proven absolutely that these lateral outgrowths in *C. fulicalis* are functional gills and as respiratory organs they might be questioned completely. Such a view would render similar organs in other orders of insects devoid of respiratory significance and, pending further investigation, it would seem that circumstantial evidence points rather definitely to the conclusion that these organs do function in respiration

Judging entirely from the structure of these gills, a contrast appears between the *Nymphula* group and *Cataclysta* which may indicate difference in degree of efficiency. In the former, the larger number of gills, the rather spacious gill-cavities, the thin gill-walls, and the profuse tracheation all suggest an efficient equipment. In the latter, however, with only about one hundred and twenty gills, with the very small gill-cavities connected

with the haemocoele by still smaller lumina, with no traces of tracheation, and with the conspicuously thick gill-walls, the effectiveness of the system seems much smaller.

The presence and absence of gills within the genera *Nymphula* and *Cataclysta* and the existence of distinctly different gill types in these two closely related genera give added support to the theory of the independent origin of the various aquatic insects, emphasizing the fact that in these animals types of adaptation and genetic relationship may have no close correlation.

The Spiracles and Connecting Tracheae

The secondarily acquired nature of aquatic habits and structures naturally directs attention to the character of the spiracles. In the gilled forms, is the gill system superimposed upon an unmodified holopneustic tracheation, or have modifications occurred leading towards suppression of the spiracular equipment? In the non-gilled forms which lead a submerged existence, has the characteristically terrestrial holopneustic tracheation been modified? A common statement appears in the literature to the effect that many nymphs and larvae living in water have apneustic tracheation, breathing directly through the skin or by means of gills. It is also pointed out that between the completely apneustic and the typical holopneustic tracheation a variety of intermediate stages exists. The gilled larvae of certain *Nymphula* species have been described as having apneustic tracheation in which the spiracles are closed, and the spiracular branches (stigmatal branches) have become solid cords. The writer has searched in vain for any thoroughgoing morphological work bearing on this subject. Among workers who have studied Old World species of *Nymphula* three of the most recent might be mentioned. Rebel (1899) made some studies on *Nymphula* (*Paraponyx*) *stratiotata* and states that the tracheation is apneustic. Portier (1911) in a voluminous paper dealing with several aquatic insects, carried on some physiological experiments with larvae of *Nymphula stratiotata* and claims to have shown that (a) larvae submerged for five minutes in olive oil colored with alcanine showed no oil in the tracheae; (b) that under the binocular microscope the spiracular trunks did not have the aspect characteristic of air filled tracheae but looked like heavy cords; (c) that larvae suffered no effects from submergence in oil, but a small geometrid larva so treated became inert and oil was found in its tracheae; (d) that larvae were perfectly normal after fifteen minutes submergence in soapy water, but the geometrid larva so treated became apparently dead in three minutes; (e) that larvae immersed in olive oil, ether, and alcanine became aenethetized after one minute, but microscopic examination showed no penetration of the colored oil, recovery occurring when returned to water; (f) that larva treated as in (e) for

twenty hours did not show the tracheal system invaded; (g) that a larva placed under reduced air pressure showed bubbles of gas gradually form on the surface of the body where the integument is thinnest but none formed about any of the spiracles. From these experiments and without morphological confirmation Portier concluded that the spiracles of the larva of *N. stratiotata* are closed and functionless. Wesenberg-Lund (1913, p. 126) states that in *N.* ("*Paraponyx*") *stratiotata* the spiracles are functionless but no evidence is given in support of this conclusion.

Observations and Experiments of Nymphula Maculalis

Attention was first directed to the spiracles by evidence that these larvae can exist out of water for considerable periods of time. While it is common for the pupa to be formed on the lower, submerged surface of a water-lily leaf, the full-grown larva sometimes crawls out of the water, onto the upper leaf-surface and there forms the pupa. In order to construct the silken covering, tie down the case to the leaf, and transform into a pupa, a considerable period of time must be spent out of water. Mature larvae placed in containers without food and with little or no moisture often lived from four to eight days. Larvae, in containers with food material and just enough water to keep the surrounding air moist, were allowed to gradually dry up. In such cases the gills became dried and black, but the larvae lived about fourteen days. It is thus evident that these caterpillars can exist for days apart from water and respire by means other than gills. Furthermore, it seems unlikely that this can be accounted for on the basis of cutaneous respiration. These results led to a critical morphological examination of the spiracles and their connecting tracheae in *N. maculalis*, a study which was later extended to include all of the strictly aquatic caterpillars available for examination.

Morphology of the Spiracles and Connecting Tracheae

A detailed morphological study of the spiracles and their connecting tracheae was made on serial sections, cut six to seven microns thick, and double stained. Both transverse and longitudinal sections were used and all critical points determined with a modern monobinocular microscope equipped with a 1.9 mm. fluorite oil immersion objective. Since the spiracular aperture may be narrower in one dimension than the other, the long dimension being transverse to the long axis of the body, measurements on transverse sections might lead to error if not checked on longitudinal sections. In all such cases the measurements were made from the edges of the opening, not including accessory structures, and in such a way as to give an average of the two principal diameters of the aperture. On the other hand, the connecting tracheae are practically cylindrical, thus making it possible to record measurements from any section passing

through the center of the lumina. Mature larvae were usually used, although sections of earlier instars were examined from time to time.

In this connection it should be pointed out that, as usual in lepidopterous larvae, the spiracles on the meso- and metathorax are absent in all of the species examined. In the following tables percentage of decrease, wherever expressed, is calculated by using as the standard of comparison the dimensions of the largest spiracle and connecting trachea (usually those of the second abdominal segment) of the series. It must also be understood that this is merely a convenient way of comparing the degree of reduction of the other spiracles and tracheae and is not intended to imply that even the largest may not have undergone some reduction themselves. Since there is no way of determining the diminution of the largest spiracle, if it has been reduced, it would not be possible to express the *true amount of reduction* of the other spiracles on the same individual.

Nymphula maculalis

Examined externally, under magnification, nine pairs of spiracles are observable on segments 1, and 4–11. All are minute and inconspicuous except those on 5, 6, and 7 which are distinctly larger. The following table comprises one set of diameter measurements which is representative of all others made in this work:

Segments	Spiracles	Lumen of Spiracular Tracheae	Percentage of Decrease	
			Spiracles	Tracheae
1	0.0050	0.0011	85.5	96.0
2	.....	.....	.....	.....
3	.....	.....	.....	.....
4	0.0051	0.0011	85.2	96.0
5	0.0345	0.0276	0.	0.
6	0.0322	0.0253	0.66	0.8
7	0.0322	0.0184	0.66	33.3
8	0.0119	0.0011	65.5	96.0
9	0.0085	0.0017	75.3	93.8
10	0.0068	0.0017	80.2	93.8
11	0.0051	0.0013	85.2	95.2

The Larger Spiracles and Connecting Tracheae

In the larger spiracles the outer margin of the aperture bears a complete set of elongated, closely set, chitinous spines (Pl. X, fig. 1) which have a rich yellow color when viewed under magnification. These spines form an almost continuous marginal guarding device, the free ends converging so that the form of the whole is that of a truncated cone. The free ends of the spines mark the periphery of an aperture which is much

smaller than the spiracle itself. The external cuticula extends into the lumen of the spiracular trachea, lining it for the entire length. However, certain modifications appear chief among which are the distinct reduction in thickness and the numerous filiform chitinous projections which extend into the lumen. A continuation of the hypodermis of the body-wall constitutes the major part of the wall of the spiracular trachea and shows no significant changes in structure.

The short connecting trunk is terminated at its ental end by a well-developed closing apparatus (Pl. X, fig. 4), composed essentially of a closing bow, a closing band, a closing lever, and an occlusor muscle. The closing bow is a chitinous, crescentic band (Pl. XI, fig. 7) lying in the lining of the lumen and extending through one-half of the total circumference. From one end of the closing bow a similar band continues around to a point about opposite the middle of the closing bow where it meets the end of the closing lever. The closing lever is located at right angles to the lumen and projects radially for its entire length, covered by an extension of the hypodermal wall. A short, broad occlusor muscle extends from the end of the closing lever diagonally to the free end of the closing bow. It thus appears that the chitinous band formed in this way is absent for about one fourth of the circumference of the lumen, thus failing to form a complete ring. Thus far it has been impossible to determine the exact relation of the chitinous parts of the closing apparatus to each other. Studies were made using thin serial sections in all the principal planes; also by dissecting out a portion of the body of the larva containing a large spiracle and its related tracheal parts, placing them on a slide in strong potassium hydroxide solution and boiling by holding the slide over a small flame. By the use of this last named method the soft parts were all removed leaving only the chitinous portions. Difficulties in tracing out certain minute portions of this closing apparatus have not been entirely overcome either by the kind of preparation or by high magnification. It appears, however, that the chitinous band, forming the closing lever and the closing bow, is one continuous structure.

From the outermost end of the closing lever a long muscle band extends diagonally to the body-wall. On the opposite side a similar muscle extends from a point near the origin of the occlusor muscle diagonally to the body-wall. Beyond the closing apparatus the lumen opens directly into that of the main longitudinal tracheal trunk.

The Smaller Spiracles and Connecting Tracheae.—The spiracles on segments 1, 4, 8, 9, 10, and 11 show a marked reduction in size, in fact, they are so small that magnification is necessary to locate them definitely. Structurally, these spiracles and their connecting tracheae differ markedly from those on 5, 6, and 7. At the margin of each spiracle there appears, instead of a thick set crown of chitinous spines, a solid, continuous, chiti-

nous rim (Pl. X, fig. 3) which projects distad from the body-surface. It would appear that in the process of reduction the spiny crown of the original large spiracles became fused into one continuous margin. At the base of the tiny cup thus formed the lumen becomes reduced to an extremely fine canal which extends without change in diameter to the closing apparatus located well within the haemocoele. This canal is lined throughout by a uniform, thin extension of the external cuticula, but the hair-like projections characteristic of the large spiracular trunks are here entirely wanting. As will appear in the table, the lumen is very minute, but by means of thin, serial sections and high magnification it has been possible to demonstrate that it is *open* throughout its course. The bulk of the wall of the spiracular trachea is composed of an extension of the hypodermis of the body-wall, but it also has become reduced, being about one-half the thickness of the same layer in the larger spiracular tracheae. The connecting trachea has not changed in length and terminates in a closing apparatus similar to that described for the larger spiracular tracheae, except that it also has become considerably reduced in size. All of the parts are represented, however, and the whole closing contrivance has every appearance of being completely functional.

It also appears that reduction in the small spiracles is not uniform, but gradually increases posteriorad. This, however, does not seem to hold for the connecting tracheae.

Nymphula obscuralis

An examination of *Nymphula obscuralis* showed a condition very similar to that in *N. maculalis*. While the following diameter measurements were taken from a single mature larva, they are representative of those for other larvae. The structural features of the spiracles, the connecting

Segment	Spiracles	Lumen of Spiracular Tracheae	Percentage of Decrease	
			In Spiracles	In Tracheae
1	0.0074	0.0005	66.6	97.3
2	.....	.....	.....	.....
3	.....	.....	.....	.....
4	0.0074	0.0005	66.6	97.3
5	0.0222	0.0185	0.0	0.0
6	0.0222	0.0185	0.0	0.0
7	0.0222	0.0185	0.0	0.0
8	0.0074	0.0024	66.6	87.0
9	0.0074	0.0017	66.6	90.8
10	0.0074	0.0017	66.6	90.8
11	0.0074	0.0005	66.6	97.3

tracheae, and the closing apparatus, are also so similar that no description is necessary here.

Cataclysta fulicalis

In *Cataclysta fulicalis* the spiracles and the connecting tracheae differ from those of the gilled Nymphula caterpillars in that no reduction of any kind appears, all being of practically uniform size and structure. Some variation occurs but it is slight and apparently insignificant. The circle of guarding spines at the outer periphery of the spiracle is less convergent (Pl. X, fig. 2) than in the gilled *Nymphula* larvae, thus forming a wider aperture. Structurally, the spiracles, connecting tracheae, and the closing apparatus are similar to those of the gilled *Nymphula* group, slight but inconsequential deviations being present. The following table includes a typical set of diameter measurements made on one specimen:

Segment.	1	2	3	4	5	6	7	8	9	10	11
Spiracle..	0.026	.....	.....	0.029	0.028	0.028	0.03	0.035	0.031	0.031	0.031
Lumen of trachea.	0.03	.....	.....	0.033	0.043	0.035	0.035	0.033	0.040	0.038	0.038

When all measurements were averaged, the spiracles and their connecting trunks were found virtually uniform in size. The larvae of *Cataclysta fulicalis* have thus acquired a system of gills without accompanying changes in the spiracular system, the latter being as completely open morphologically as any terrestrial caterpillar.

Nymphula obliteralis

The spiracles and connecting tracheae are distinctly open and show no evidence of definite reduction. A certain variation appears, as will be noted in the following table, but even the smallest found is far above the corresponding reduced structures in *N. maculalis* and *N. obscuralis*. In the following representative diameter measurements taken from the record of one mature specimen, nothing is especially noteworthy except the large spiracle and connecting trachea on the fourth segment. Whether the difference in size is an evidence of a slight reduction of spiracles and connecting tracheae in the posterior segments is uncertain. The principal features of the closing apparatus are shown in figure 8.

Segment.	1	2	3	4	5	6	7	8	9	10	11
Spiracle..	0.032	.....	.....	0.044	0.04	0.04	0.04	0.032	0.032	0.036	0.04
Lumen of trachea.	0.028	.....	.....	0.064	0.028	0.028	0.024	0.020	0.020	0.020	0.036

Nymphula sp.

A non-gilled form, distinctly different from any other used in this work but whose specific identity is unknown, was examined in this connection. Sections showed all of the spiracles to be distinct, well-developed, open, and approximately uniform in structure and size, the same being true of the connecting tracheae. Average measurements for the whole series are as follows, the extreme variations deviating very little from the average: Diameter of the spiracles, 0.05569; diameter of the lumina of the tracheae, 0.0527.

Experiments

In order to demonstrate experimentally the open condition of the spiracles and connecting tracheae and check the morphological results, certain experiments were made on the caterpillars *N. maculalis*. Larvae dropped into hot water invariably give off one or more bubbles of gas from the large spiracles on segments 5–7, thus showing definitely the open condition of these spiracles and their connecting tubes. No gas was given off from the reduced spiracles, but it does not follow that such failure is due to complete closure since the amount of gas in the tiny lumina is extremely slight and the expansion of the heated gas in the longi tudinal tracheal trunks would be more likely to be released at the larger and more open spiracles on segments 5–7.

It was found that the larvae of *N. maculalis* could live in commercial kerosene for 6–7 hours. They were then submerged in kerosene colored with Sudan III. Since the translucency of the body-wall made it possible to trace much of tracheal system, it was easy to examine the spiracular connections at any time and to follow the entrance of the colored liquid into the larger spiracles. Positive evidence that these spiracles are morphologically open was thus repeatedly secured. This penetration, into the large spiracles, occurred within an hour, but entrance into the smaller ones was much slower although ultimately the colored liquid was observed in some of the connecting tracheae.

Discussion

It thus appears, at least in *Nymphula maculalis*, *N. obscuralis*, and *Cataclysta fulicalis*, that in spite of the gill development, the tracheal system of the larvae is morphologically open. The reduction of certain spiracles and their connecting tracheae in the first two is definite and striking but has not progressed to the place where the lumina and apertures are completely closed. Since the previous work on this subject has been done on unavailable foreign species it is not possible, on the basis of the present work, to absolutely refute the statements made in the literature, but the writer is inclined to suspect strongly that what has been found

in *N. maculalis* and *N. obscuralis* is likewise true of *N. stratiotata* and other foreign gilled representatives of that genus. Portier's results (1911), secured as they were without any attempt at critical morphological work, cannot be regarded as conclusive. However, it is not inconceivable or impossible that certain species might have progressed to the point of closing the spiracular system but if such a condition does exist it should be demonstrated more convincingly than has heretofore been done.

In regard to the functioning of these reduced spiracles and tracheae, no serious question can be raised concerning the larger ones on segments 5–7 in *N. maculalis* and *N. obscuralis* since their size, open character of the connecting tracheae, and structure of the closing apparatus all indicate the possibility of normal activity. In spite of the small diameter of the more reduced spiracles and tracheae, the writer has thus far found nothing which would prohibit at least a limited functioning of these organs. That air will pass through pores and tubes of smaller diameters than those of the organs under discussion is now known, and many of the very minute insects known to have a typical holopneustic type of tracheation have openings and tracheae no larger than the reduced ones of the gilled *Nymphula* caterpillars. Likewise if the minute tracheoles of the tracheal system which are less than one micron in diameter can transport atmospheric gases, failure of the reduced spiracles and connecting tracheae to function would have to be due to some feature other than the structure of the tubes themselves. That there would no difficulty in the ventilation of such a system has recently been shown by Krogh (1920a; 1920b) since in small forms diffusion alone will provide the necessary oxygen transportation, although it may be assisted by respiratory movements of the animal, if the latter are manifested.

In certain insects having apneustic tracheation the spiracles and connecting tracheae are said to be temporarily open at the time of molting. This, however, does not account for the open character of the gilled larvae of *Nymphula* since sections of specimens in various parts of the stadia involved were studied and all yielded the same result.

There seem to be no reasons for assuming that open spiracles and open connecting tracheae are necessarily inimical to larvae existing in water since certain well known forms, as for example, *Bellura melanopyga*, have complete sets of open spiracles, yet are related to the water in such a way that most or all of these organs are submerged for long periods of time. It is not unlikely that still other aquatic larvae, thought to have true apneustic tracheation, will be found to possess morphologically open spiracles.

What part these open spiracles play in the life of the forms involved is difficult, at present, to specify. As has been pointed out, the gilled *Nymphula* larvae can pass extended periods of time out of water, at least

during the last larval stadium. This indicates functioning of the spiracles, since it is unlikely that the requisite amount of oxygen could be secured by cutaneous means alone, especially after the surface of the body became dry. In regard to submergence, it is possible that a provision against penetration of water into the tracheal system is afforded in the combination of structures present. The marginal crown of guarding spines or their derivatives, if hydrofuge in character, may constitute an efficient protection. It is also possible that the well developed closing apparatus plays some part in this connection.

General Considerations

From the point of view of the respiratory mechanisms involved, the true aquatic Lepidoptera comprise a heterogeneous assemblage, including those, on the one hand, which have made no morphological advance towards the aquatic life, and those on the other hand, which manifest highly developed morphological adaptations of an aquatic sort. These adaptations involve, in the most complex type, the addition of structurally complex gills, and the marked reduction in size of spiracles and connecting tracheae. It should be noted that apparently no advantage has accrued to the possessors of the complex adaptation since in all of the situations examined by the writer the non-gilled larvae, having the unmodified tracheal system of a terrestrial type, have had every appearance of being as successful in the aquatic medium as their more specialized relatives, often existing in identically the same environment and offering an interesting parallel in the solution of the same problems by very different means. There would seem to be a considerable advantage in the possession of over 400 gill-filaments and a set of reduced spiracles, particularly in those cases where the size of the body proper is virtually that of the associated non-gilled forms. As previously mentioned, the structure of the gills in *Nymphula* is such that it seems almost inconceivable that they do not function as true respiratory organs. In a recent paper, Fox (1921) claims to have demonstrated that in a certain chironomid larva, oxygen is not taken up by the ventral blood gills; that the anal gills take up less than the corresponding area of the body-surface; and that most of the oxygen is received through the body-wall in general. These surprising results require confirmation and at present need not be regarded as serious ground for questioning the function of the gills in other insects.

On the basis of structure alone it might appear that the small blood gills of *Cataclysta* represent a more primitive stage in the development of aquatic respiratory adaptation than that represented in the gilled larvae of *Nymphula*. However, there is no indication that any of the *Nymphula* species have ever had blood gills only. The evolution of these larval

adaptations has apparently been a sporadic phenomenon with the extremes sometimes occurring within the confines of a single genus.

Summary

1. Fundamentally, aquatic larvae of the genus *Nymphula* have retained the original terrestrial type of body tracheation in practically unmodified form. The tracheation of the gills has been superimposed upon the terrestrial type with minimal change to the latter, and the non-gilled larval type is doubtless the older one phylogenetically.

2. Gills in the aquatic Lepidoptera are all hollow outgrowths of the body-wall, the cavity being in direct communication with the haemocoele. All of the layers of the body-wall are represented but in reduced thickness, maximum diminution appearing in the dermis.

3. In all of the gilled larvae of *Nymphula* examined, the gill-cavity contains both an elaborate set of tracheae and tracheoles and a considerable quantity of body fluid, thus constituting a combination tracheal-blood gill. In the larvae of *Cataclysta fulicalis* the gills have no traces of tracheae and are thus blood gills only.

4. In the non-gilled larvae of *Nymphula* and the gilled larvae of *Cataclysta fulicalis* the tracheation is typically holopneustic, no reduction of any significance appearing either in the spiracles or their connecting tracheae.

5. In gilled larvae of *Nymphula*, a distinct reduction appears in the spiracles and their connecting tracheae on segments 1, 4, 8, 9, 10, and 11, those on segments 5, 6, and 7 being much larger and having undergone less reduction.

6. Morphological and experimental studies on gilled *Nymphula* larvae have shown that in spite of the striking reduction of some of the spiracles and connecting tracheae, the tracheation is still holopneustic, all spiracles and tracheae being morphologically open with nothing to indicate that they are functionless. While gilled representatives of foreign species of this genus have not been available, it is very probable that the statements in the literature to the effect that they have a closed tracheal and spiracular system are in error, due to insufficient study.

7. The gilled larvae of *Nymphula maculalis* may live for extended periods of time outside of water, even after the outer surface becomes dry and the gill-filaments shriveled, indicating that respiration through the spiracles is being accomplished.

8. Reduction of spiracles and possession of gills do not seem to be necessarily correlated or coexistent since in *Cataclysta fulicalis* both gills and an unreduced tracheal system are present.

9. In spite of the contrast between the gilled and the non-gilled species, the former seem to have no advantage over the latter, at least in those cases where both forms exist side by side in the same habitat.

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Abbreviations Used in Plates

bl.	blood
cl.	closing apparatus
c.t.	connecting trachea
d.	dermis
ep.	epidermis
gl.c.	gill cavity
hyp.	hypodermis
l.t.	longitudinal trachea
mu.	muscle
sp.	spiracle
t.	trachea

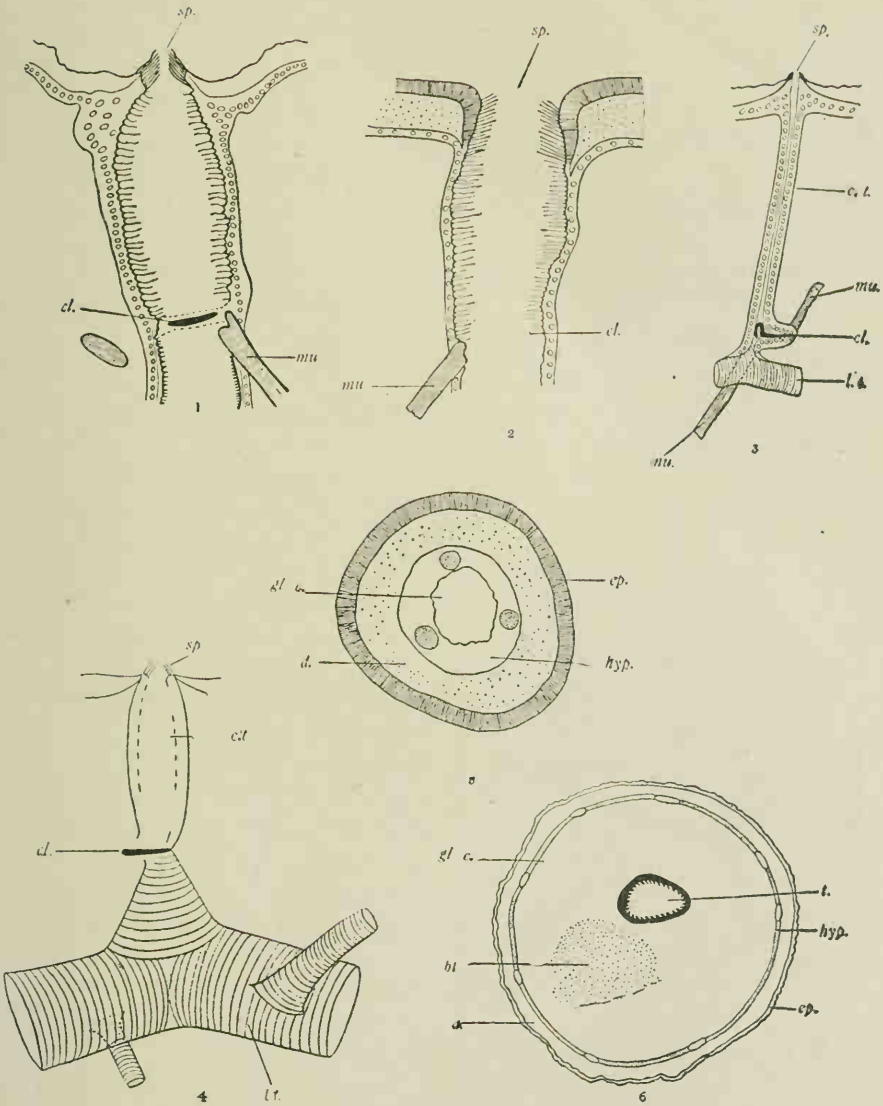


PLATE X

Fig. 1. Longitudinal section through large spiracle and connecting trachea in larva of *Nymphula maculalis*.

Fig. 2. Longitudinal section through spiracle and connecting trachea in larva of *Cataclysta fulicalis*.

Fig. 3. Longitudinal section through reduced spiracle and connecting trachea in larva of *Nymphula maculalis*.

Fig. 4. Drawing of caustic potash preparation of large spiracle and connecting trachea in larva of *Nymphula maculalis*.

Fig. 5. Transverse section of gill-filament in larva of *Cataclysta fulicalis*.

Fig. 6. Transverse section of gill-filament in larva of *Nymphula maculalis*.

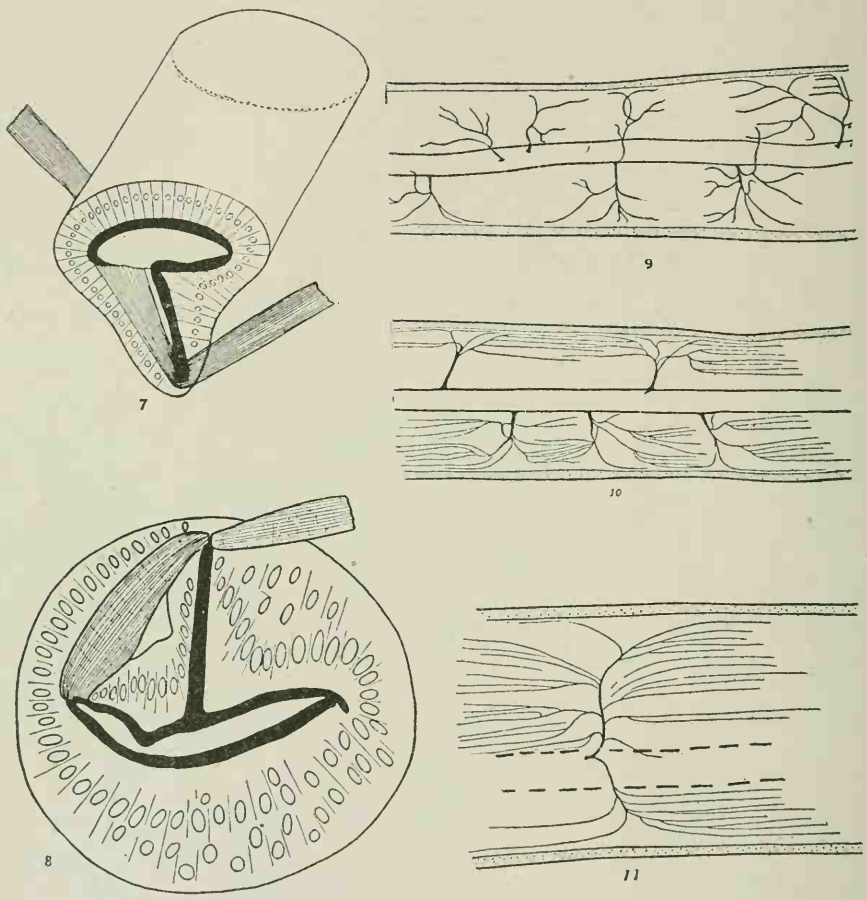


PLATE XI

Fig. 7. Transverse section at ental end of connecting trachea for large spiracle in larva of *Nymphula maculalis*, showing structure of closing apparatus.

Fig. 8. Transverse section at ental end of connecting trachea for spiracle in larva of *Nymphula obliteralis*, showing structure of closing apparatus.

Fig. 9. Camera lucida drawing from living larva of *N. maculalis* showing tracheation of gill-filament as it appears under low magnification.

Fig. 10–11. Camera lucida drawings from living larva of *N. maculalis* showing distribution and arrangement of finer tracheoles of gill-filament as they appear under high magnification.

DEPARTMENT OF METHODS, REVIEWS, ABSTRACTS, AND BRIEFER ARTICLES

DICHROMATIC ILLUMINATION FOR THE MICROSCOPE

By
Leon Augustus Hausman, Ph.D.

The practise of using monochromatic light in photomicrographic work is widespread. The advantages in the use of such light in conjunction with ocular microscopic examination seem to be less appreciated; while the use of dichromatic illumination, such as that described in this paper, the writer believes to be new.

In using monochromatic light, i.e., light of a given color or wave-length for microscopic examination, the purpose is three-fold: (1) to increase the resolving power of the objective, (2) to secure greater contrast between different parts of the specimen, and (3) to afford relaxation for the eyes. Abbe's formula for ascertaining the resolving power of an objective is to multiply the numerical aperture of the objective by twice the number per inch of the waves of the light employed. Hence it follows that the shorter the wave length of the light, the greater the resolution of the objective. This has an application of appreciable value in connection with the visibility and sharpness of focus of minute objects which seem to lie just upon the border-line of vision. Moreover after working long with white light, green light affords a grateful relaxation to the eyes.

The microscope screen (S, Fig. 1) devised by the writer, has been used with success both for monochromatic and dichromatic illumination. With the former type of illumination the color filter is inserted in the slide (C1, Figs. 1 and 2) and the light obtained from the arc-lamp (A1, Figs. 1 and 2).

For the examination of minute structures in the protozoan cell, and of the pigment granules in the cortex of mammalian hairs, green or blue illumination was found to be excellent. The former is more restful to the eyes, especially when making protracted examinations. It is, moreover, of greater luminosity, and hence permits of greater ease in focussing the specimen. In the examination of protozoa the writer's practise is to focus the object by the green light, and then exchange this for the blue. Violet light was found unsatisfactory, because of its lack of luminosity. Certain

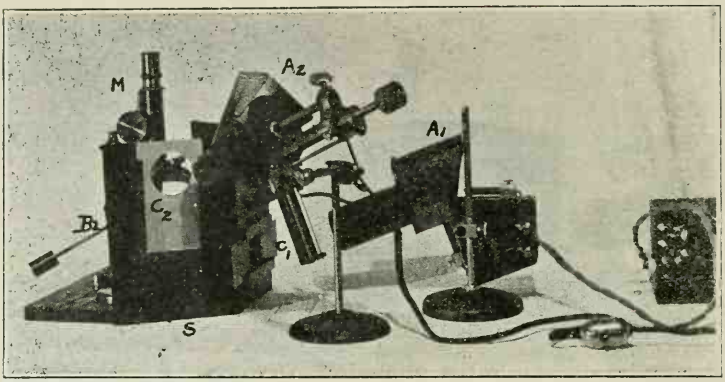


FIG. 1

Assemblage of apparatus for dichromatic illumination. Microscope (M) in position behind the screen (S), which bears slides (C_1 and C_2) for supporting the color filters. Illumination of the object above and below is secured from the two arc lamps (A_1 and A_2).

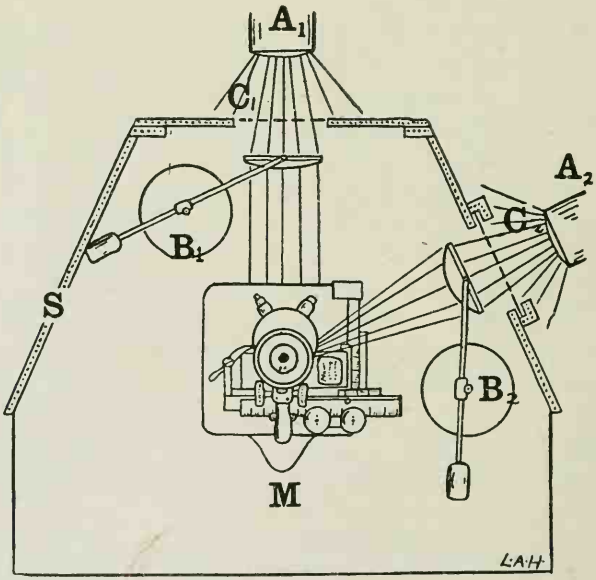


FIG. 2

Diagrammatic view of apparatus for dichromatic illumination, from above. A_1 and A_2 arc lamps, for transmitted and reflected light respectively; B_1 and B_2, plano-convex condensers; the former to deliver practically parallel rays to mirror, the latter to illumine object from above; C_1 and C_2, apertures for color filters; M, microscope. The screen (S) is stippled.

stained structures show up well with monochromatic light, particularly if the color used be complementary to the one used as stain.

By employing a new type of illumination, i.e., dichromatic, it is possible by means of the illumination alone to invest certain portions of the specimen with one color, and other portions with another, for the purpose of bringing out thus by contrast the forms or relationships of the two. The principle of dichromatic illumination is to illuminate the microscope field and the transparent portions of the specimen with one color, by means of transmitted light, and the more dense, or opaque portions of the specimen with another color, by means of reflected light, and to secure the maximum contrast between the structures so illuminated by using complementary colors.

Such a result has been secured by means of the apparatus shown in Figs. 1 and 2 (whose letterings are similar). The microscope (M) is sufficiently protected from all rays of light save those desired, by the screen (S), which bear two slides (C1 and C2). In these slides are apertures wherein can be placed color filters. Filter C1 delivers to the microscope mirror light of one color for illuminating the field and the transparent portions of the specimen, and filter C2 delivers light of another color which is focussed upon the specimen from above. The transparent portions of the specimen are therefore viewed by transmitted light of one color, the opaque portions by reflected light of another color, and the translucent portions by a combination of these two. Slide C1 bearing its filter can be moved laterally, and slide C2 vertically. The positions which the filters can be made to occupy, together with the various positions of the microscope within the screen, make it possible to secure light from any of the angles useful in microscopic work. Illumination is furnished by two movable arc-lamps, A1 for delivering light to the mirror, and A2 for lighting the specimen from above.

Within the microscope screen two movable plano-convex condensers are used; one of long focus (B1), for delivering to the mirror virtually parallel rays; the other (B2) of short focus for condensing the light upon the specimen from above.

With such an apparatus the writer has secured good results in the examination of mammalian hairs, for the detection of delicate scalation. With further experimentation the use of such lighting may possibly be widened.

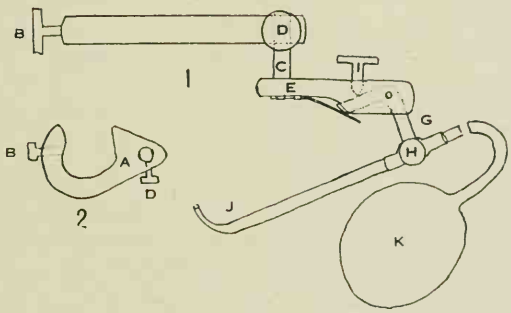
Color filters for use in photomicrographic work can be purchased and used in the screen. Good filters can be made, however, in the laboratory by developing out unexposed lantern slides, or dry plates, and then staining the clear gelatin film with the desired colors. Care must be taken to secure color filters that will allow only one color of light to pass through them, i.e., they must furnish, as nearly as possible, monochromatic light. Some

of the stains, in aqueous solution, which have been recommended for this purpose are: (1) Yellow—a saturated solution of picric acid. This absorbs almost completely the blue and violet end of the spectrum. (2) Green—methyl green. The absorption spectra varies with the depth of the color. (3) Copper sulphate. This absorbs the red almost entirely. Methyl blue is fairly good. (4) Red—Safranin.

The procedure in making color filters from lantern slide plates is to develop and fix the unexposed plates and then to allow the gelatin film to soften by placing the plates in a bath of slightly warm water, say 80 degrees F. for a few minutes. They may then be removed to baths of the different stains, and allowed to soak for an hour or so, or until the gelatin is evenly stained. It was found best to make up a series of saturated aqueous solutions of the stains, and from these gradually to increase the depth of color of the various baths until the desired depth was secured in the gelatin film. The optimum for color filters is the greatest depth of color which one can use and still secure sufficient luminosity for good focus. The depth of the color of the filter giving the best results will depend upon the brightness of the illuminant. This latter should give a strong, white light. Such a light, with a spectrum very much like that of sunlight, can be obtained from the electric arc.

A MODIFIED BARBER PIPETTE

The writer has been much interested in the various modifications of the Barber pipette. One, which seems to be more simple than any here-to-fore described, has been in use in these laboratories since 1916. It seems worth while to describe it. The device consists of a bar A (side view—figure 1, top view—figure 2) fashioned to fit partly around the objective and fastened to it by the thumb screw B. The rod C passes thru this bar and is held in position by the thumb-screw D. The bar E is fastened to the rod C by a screw which also passes thru the spring F. The part G is composed of a ring thru which a glass rod passes (the latter



held in position by the thumb screw H) and an angular piece with pivots at the angle. There is a thumb screw I which controls the upward and downward movement of the point of the pipette J. The camera bulb K completes the apparatus. This device, minus the hollow tube and the camera bulb, was devised by the late Dr. J. J. Wolfe for use in the picking up of diatoms by adhesion. The hollow tube and camera bulb were added by the writer for picking up copepods. This device later became very useful in the segregation of living diatoms. A mechanical stage may be used with the pipette. Altho the laboratory possesses a real Barber pipette, this simpler apparatus is preferable when diatoms or larger organisms are to be segregated.

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CLEANING SLIDES AND COVERS FOR DARK-FIELD WORK

As particles of dirt show with the same brilliancy as the bodies one wishes to study, the slides and covers must be made very clean.

One of the most satisfactory ways of cleaning the slides is that suggested by Stitt in his book on the blood etc., p. 235, 5th Ed. near the top. I have found the modification here given very satisfactory:

(1) 5 grams of powdered Bon Ami are mixed with 100 cc of water and thoroughly shaken up.

(2) New slides and covers are put into this mixture and well wetted, then they are taken out and stood up on blotting paper to drain and dry. The method answers well also for the final polishing of cover-glasses that have been cleaned in acid-dichromate mixture, and for used slides that have been cleaned in any approved method.

(3) Whenever one wants a slide or a cover one of those on which is the dried Bon Ami is taken and wiped with a clean piece of gauze. It is astonishing how quickly and well the cleaning can be done in this way. Very few of them show any particles of dirt with the dark-field miscroscope.

(4) Cleaning the used slides and covers.—Hot water is allowed to flow on the slide to wash off the oil, then the cover is removed and put into cleaning mixture (Sulfuric acid and dichromate). The slide can usually be well cleaned with the Bon Ami.

S. H. Gage.

PROCEEDINGS OF THE AMERICAN MICROSCOPICAL SOCIETY

Minutes of the Toronto Meeting

The 40th annual meeting of the American Microscopical Society was held in affiliation with the American Association for the Advancement of Science at Toronto, Canada, December 29, 1921.

In the absence of the President, Frank Smith, and both Vice-Presidents, Professor Albert M. Reese acted as Chairman.

The report of the Treasurer for the year 1921 was read by the Secretary and referred to an Auditing Committee composed of Profs. R. J. Pool and R. H. Wolcott.

The report of the Custodian was read by the Secretary and referred to an Auditing Committee composed of Messrs. Edw. Pennock and F. E. Ives.

The meeting voted to send congratulations to the Custodian, Mr. Magnus Pflaum on the growth of the Spencer-Tolles Fund.

The Secretary presented a general report on the affairs of his office.

The following officers were nominated and elected for the constitutional periods: President, Dr. N. A. Cobb, Bureau of Plant Industry, Washington, D. C.; First Vice-President, Professor E. M. Gilbert, University of Wisconsin; Second Vice-President, Professor Z. P. Metcalf, North Carolina State College of Agriculture and Engineering; Custodian, Mr. Magnus Pflaum, Philadelphia, Pa.

Dr. B. H. Ransom, Bureau of Animal Industry, Professor Chancey Juday, University of Wisconsin, and Professor George R. La Rue, University of Michigan, were chosen as the elective members of the Executive Committee for 1922.

Adjourned.

P. S. Welch, *Secretary.*

Reports of the Treasurer and Custodian

Because of unavoidable delays, the reports of the Treasurer and the Custodian can not appear until the April number.

5-8

TRANSACTIONS

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TRANSACTIONS
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ON THE PROTOZOA PARASITIC IN FROGS*

By
R. Kudo
University of Illinois

Probably no other animals have been for many years more favorite objects of studies by zoologists than the frogs. The amphibians have been examined by several Protozoologists and we know at present a considerable number of Protozoa of a great variety parasitic in frogs from various parts of the world.

Numerous publications dealing with the protozoan parasites of frogs have been issued by authors of several nationalities. Aside from the papers by North American workers such as Ohlmacher (1893), Whinery (1893), Gurley (1894), Stebbins (1904, 1905), Lewis and Williams (1905), Metcalf (1909) and Swezy (1915, 1915a), a large majority are widely scattered in various periodicals, and are not always easily referred to. Undoubtedly this hardship concerning literature prevented the students in Zoology from taking advantage of the material. If one possesses therefore brief accounts of the Protozoa commonly found in frogs, hundreds of which are sacrificed yearly by students in Zoology and by special investigators, one can utilize both material and time in carrying out observations upon these interesting Protozoa.

The present paper is an attempt to meet this need. It deals with my observations on the Protozoa parasitic in North American frogs which I have examined during the last two years, together with the description of methods of observation, and with brief review of and reference to the works of the previous investigators on the subject.

The following six species are described in order:

1. *Entamoeba ranarum* from the intestine
2. *Leptotheca ohlmacheri* from the kidney
3. *Haemogregarina* sp. from the blood

*Contributions from the Zoological Laboratory of University of Illinois. No. 199.

4. *Trypanosoma rotatorium* from the blood
5. *Trypanosoma parvum* nov. spec. from the blood
6. *Opalina* sp. from the intestine

I *Entamoeba ranarum* (Grassi) Dobell 1908

Habitat.—In the large intestine of *Rana temporaria*, *R. esculenta*, *R. clamitans* and *Bufo vulgaris*. Dobell (1909) saw that about 23% of *Rana temporaria* in Cambridge and Munich, were infected. I have seen a number of amoebae whose characters agree on the whole with those described by Dobell for *Entamoeba ranarum* in one out of 14 individuals of *Rana clamitans* from New York in August of 1920. In *Rana pipiens* which I have studied in 1920 and 1921 at Urbana, Illinois, I did not observe any host individual that harbored the organism. This of course does not mean its absence in a frog of this species, since I have not examined them as thoroughly as I did in the case of *Rana clamitans*.

Historical.—Lieberkühn (1854) probably noticed the Amoeba in the intestine of the frog which he studied. Grassi (1879) examined and named it *Amoeba ranarum*. Dobell (1909) found an Amoeba in the frogs of England and Germany, and studied them in detail. Quite recently, the same author (Dobell, 1918) states that although the Amoeba resembles closely morphologically to *Entamoeba histolytica* of human intestine, they are distinct species. I have met with apparently the same Protozoon but once, and could not carry observation concerning its development.

Distribution.—Europe and North America.

Methods of observation.—A portion of the large intestine of a frog is cut into small pieces in physiological salt solution on a cover-glass, made an ordinary fresh preparation and observed. The organism may live for several hours. The general appearance, changes in form of the body through the formation of pseudopodia and the structure of the protoplasm can be studied. To make permanent preparations, make smears on slides or cover-glasses and fix them with hot sublimate-alcohol-acetic mixture (2 parts of saturated aqueous solution of corrosive sublimate, 1 part of absolute alcohol and 5% of glacial acetic acid) for about 20 minutes. The smears are then immersed for about 15 minutes in a weak iodine alcohol (50%) and then transferred into a plain alcohol to remove the iodine. Staining with Delafield's haematoxylin, Heidenhain's iron haematoxylin or Dobell's alcoholic haematein, brings out satisfactory results.

Morphology.—Amoeba of moderate size. When alive, the cytoplasm is poorly differentiated into ectoplasm and endoplasm. Lobose pseudopodia are actively formed at one time from any part of the body. The peripheral portion of the cytoplasm is somewhat hyaline, while the main part of the body is granulated and contains bacteria, yeasts and other particles

present in the host intestine. The nucleus is spherical and faintly visible in living condition with an oil immersion objective. No contractile vacuole is present. Dimensions vary from 15 to 40μ in the largest diameter. When stained, the cytoplasm becomes highly vacuolated or reticulated. The nucleus is spherical and usually contains a distinct karyosome.

Figs. 1 and 2. *Entamoeba ranarum.* Fig. 1, a living individual. Fig. 2, an individual stained with Delafield. x 1500.

Development.—According to Dobell, the cysts are found in the host intestine in winter months. They are spherical, and measure 10 to 16μ in diameter with a large nucleus. The nucleus divides twice producing four daughter nuclei. Further changes are not known. Dobell suggests that the cysts serve for the dissemination of the organism. The same author (Dobell, 1918) recently found that although *Entamoeba ranarum* and *E. histolytica* can hardly be distinguished morphologically from each other, the cysts of the latter species when introduced into the intestine of tadpoles did not undergo changes which take place in their proper habitat, and concluded that these two forms should be held as different species.

II *Leptotheca ohlmacheri* (Gurley) Labbé 1899

Synonyms.—*Chloromyxum* (*Sphaerospora*) *ohlmacheri* Gurley 1893, *Leptotheca ranae* Thélohan 1895 and *Wardia ohlmacheri* Kudo 1920.

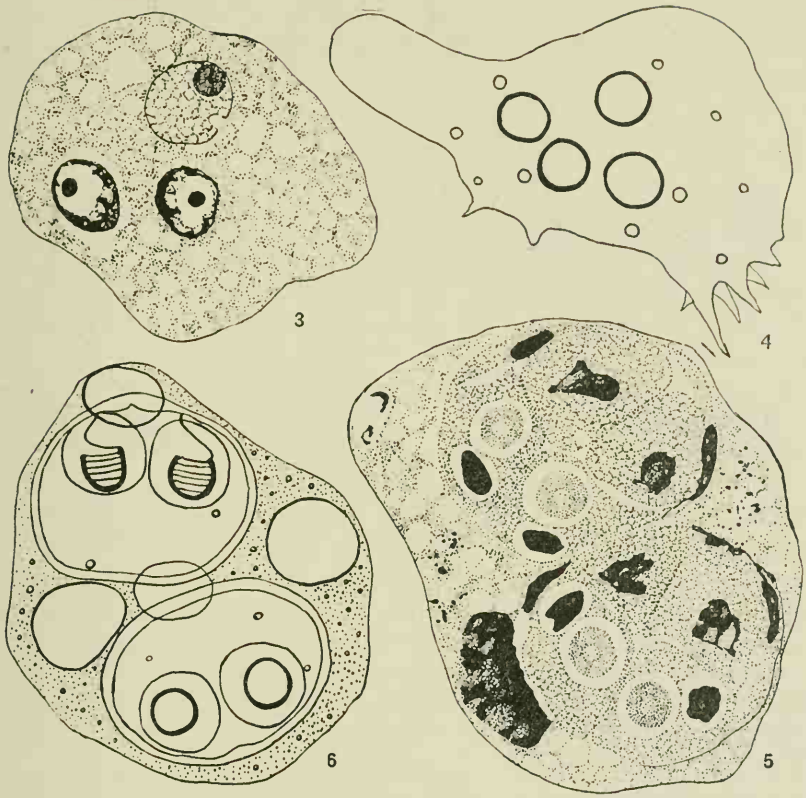
Habitat.—In the kidney of *Rana clamitans*, *R. pipiens* and *Bufo lentiginosus*. Out of 14 individuals of *Rana clamitans* examined in New York from July to September, 1920, six were infected by the parasite. Out of 24 *Rana pipiens* bought from a Chicago biological supply store and examined between November and December, 1920, ten were found to be infected by the Myxosporidian. Thélohan (1895) named a Myxosporidian which he saw in the kidneys of *Rana esculenta* and *R. temporaria*, *Leptotheca ranae*. He has not given description nor figure for it, but I am inclined to think this is probably identical with the American species.

Historical.—The spores of the Myxosporidian were first found by Ohlmacher (1893). Whinery (1893) also worked on them. Gurley (1894) summarized the observations of the two authors. Thélohan (1895) found *Leptotheca ranae* in France (?). No body seemed to have worked on the organism until 1920 when I found the vegetative stages and spores of a species what appeared to be identical with the present form. I have studied its morphology and development, the result of which will be stated elsewhere (Kudo, 1922).

Distribution.—North America and Europe (?).

Methods of observation.—When the infection is heavy, isolated spores may be found in the cloaca of the host, but the kidney must be examined for both spores and trophozoites. A small part of the kidney is cut into small pieces on a slide in a drop of physiological salt solution and made fresh preparation. In order to remove the fat globules that are usually present in smears of kidneys, one drop of weak potassium hydrate solution may be added to it. If any spores are present, they will be easily recognized under a low power due to their peculiar appearance. If the infection of the kidney is detected, hanging drop preparations or fresh preparations with physiological solution should be made immediately and sealed with melted paraffin. By using oil immersion objective, one can distinctly see the detailed structures of the spores and trophozoites of various developmental phases. To make permanent preparations, smears of variable thickness should be made. In thinly made portion, one can see the number and structure of the nuclei in well stretched trophozoites, while in thickly made part, the shape, general appearance and arrangement of nuclei and cytoplasm around them may be studied. Smears are well fixed with sublimate-alcohol-acetic mixture. For staining, besides the three methods stated for *Entamoeba ranarum*, Giemsa's method brings out beautiful results. Section preparations should also be made in order to observe the location of various developmental stages of parasites in the host organ and the relation between the parasite and host body.

Morphology.—Fully grown trophozoites are usually rounded or oval in form. Long conical pseudopodia are actively formed. Frequently the trophozoites are completely rounded without any pseudopodia. The body is colorless, granulated and extremely hyaline. The cytoplasm is indistinctly differentiated into endoplasm and ectoplasm. The endoplasm is finely granualted and contains a vegetative nucleus, two developing spores and fat globules of variable size and number. The ectoplasm is only distinctly visible where the pseudopodia are formed, the latter being usually entirely composed of ectoplasm. Before starting spore formation the trophozoites multiply by active gemmation. In almost all cases, disporous, rarely monosporous and more rarely trisporous.



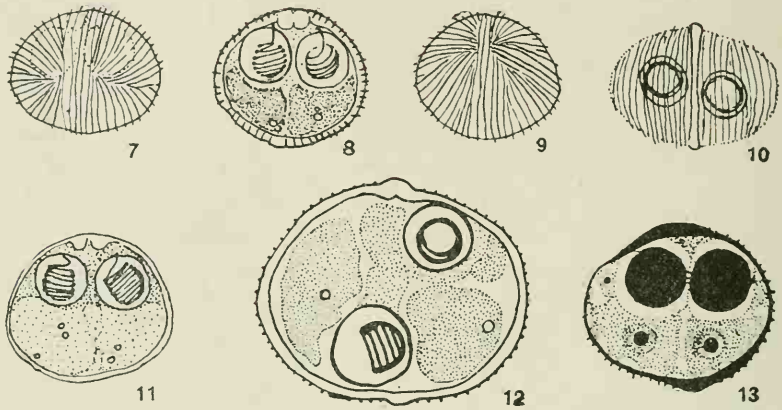
Figs. 3 to 6. Trophozoites of *Leptotheca ohlmacheri*. Fig. 3, a trinucleate trophozoite with a vegetative nucleus and two generative nuclei. A thin smear stained with Delafield. Fig. 4, a young trophozoite in which four polar capsules are being formed: fresh preparation. Fig. 5, a thinly spread trophozoite with a vegetative nucleus and two developing spores. Giemsa. Fig. 6, a rounded trophozoite with two mature spores: fresh preparation. All x 2350.

Dimensions of fully grown disporous trophozoites are 30 by 20μ, 38 by 25μ, 40 by 20μ, etc. Each spore develops independently.

Development.—With regard to the interesting development of the Myxosporidian the reader is referred to one of my papers (Kudo, 1922).

Morphology of the spore.—Oblong with its largest diameter standing at right angles to the sutural plane. Anterior end is conspicuously attenuated due to the thickening of the spore membrance at this point while the posterior end is rounded. In lateral view, it is nearly circular with a pointed anterior end. In an anterior end view, it is regularly oblong. The spore membrane is moderately thick. Sutural ridge is well marked, especially at the anterior end. The membrane is somewhat irregularly striated. Three to seven fine striae run parallel to the sutural

line on each valve and the remaining striae make somewhat similar angles with the sutural line. The striae in lateral view run parallel to one another except those on the posterior margin where a few make angles with the former. The striae on each valve vary from 25 to 35 in number.



Figs. 7 to 13. Spores of *Leptotheca ohlmacheri*. Figs. 7, 8 and 9, the upper surface, optical section and lower surface views of a normal fresh spore. Fig. 10, anterior end view of a fresh spore. Fig. 11, an optical section of a fresh spore with two large sporoplasms. Fig. 12, a fresh abnormal spore showing two sporoplasms and two capsulogenous cells, each containing a polar capsule. Fig. 13, a section through a spore showing the deeply stained polar capsules and two uninucleate sporoplasms. Section: Delafield. Figs. 7 to 11, x 1500; Figs. 12 and 13, x 2350.

Two polar capsules usually equal in size, occupy the anterior portion of the spore. The polar filament is coiled four to six times and is distinctly visible in fresh condition. It can be extruded under the action of potassium hydrate or mechanical pressure as I stated elsewhere (Kudo, 1918, 1921). Without staining the filament can be seen under a low magnification. Two independent sporoplasms occupy the extracapsular cavity of the spore, which condition is very rarely seen in Myxosporidia. They appear homogeneous in fresh state. Staining reveals that each sporoplasm contains a single nucleus. Dimensions of fresh spores: sutural diameter and thickness, 9.5 to 12 μ, breadth, 13 to 14.5 μ, diameter of polar capsules 3.5 to 4.5 μ, length of extruded polar filament, 42 to 62 μ. Those of stained spores: sutural diameter and thickness, 8.5 to 10 μ, breadth, 9 to 12 μ, diameter of polar capsules 3 to 4 μ.

III *Haemogregarina* sp.

Our knowledge of haemogregarines is still in great confusion because their development has not been studied except species occurring in reptiles. The haemogregarine described here seems to agree with the following

species: *Drepanidium magnum* Grassi et Feletti 1891, *Drepanidium krusei* Labbé 1892 and *Karyolysus clamatae* Stebbins 1905.

Habitat.—In the blood cell and plasma of *Rana clamitans* and *Rana pipiens*. I have observed it quite frequently in the last named host species. Quite frequently the frogs were found to harbor trypanosomes at the same time.

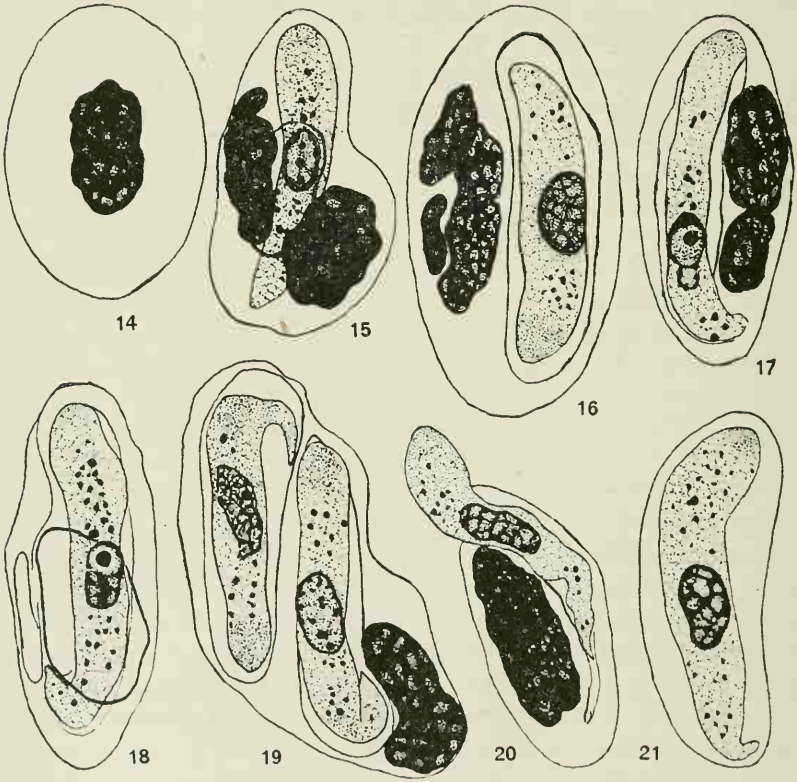
Historical.—While *Lankesterella minima* seems to have repeatedly been studied by European authors (see for instance, Hintze, 1902), the present form has been seen rarely. It seems to be this form that attracted the attention of some North American investigators such as Langmann (1898-1899) and Stebbins (1905). I have seen quite frequently the haemogregarine in frogs of New York and Illinois, but so far have not seen *Lankesterella minima*, the common European form.

Methods of observation.—Same as those stated for trypanosomes.

Morphology.—The haemogregarines found in the blood of frogs may be spoken of under two types: intracorpuscular and extracellular. The intracellular stage is cylindrical in shape, usually lying on one side of the erythrocyte and displacing the nucleus to the other side. The posterior end is usually folded up. In fresh preparation, the oblong nucleus with a distinct membrane and usually a single karyosome is seen to occupy the central portion of the body. The cytoplasm is homogeneous and contains refractive granules of variable number scattered both in the anterior and posterior regions of the body. Ordinarily there is no recognizable movements of the body except at the time prior to the emergence from the host cell in which the parasite is lodged. Around the body there is a thin but distinct membrane. When stained, the oblong nucleus assumes two kinds in appearance: one with eccentrically located karyosome and the other with chromatin granules scattered evenly on the linin network. The cytoplasm is highly reticulated and is always denser at the rounded end than at the other end. The nucleus of the host cell seems to degenerate by breaking down into a number of smaller irregular masses, and becomes faintly stained, which condition indicates that the infection probably causes some changes in the chemical nature of the nucleus of the host cell. The host cell containing the haemogregarine becomes stretched and exhibits variable shape and size. The number of parasites present in one host cell is usually one, but frequently two are present, in which case the host cell becomes greatly enlarged and deformed. The intracorpuscular forms while under observation start to turn around in the host cell, and finally breaks through the host cells. Whether this is due to the pressure caused by the cover-glass and by immersion objective or natural phenomenon cannot be determined.

The extracorpuscular stage is gregarine-like in its appearance and movements. The forms found in *Rana pipiens* and *R. clamitans* differ

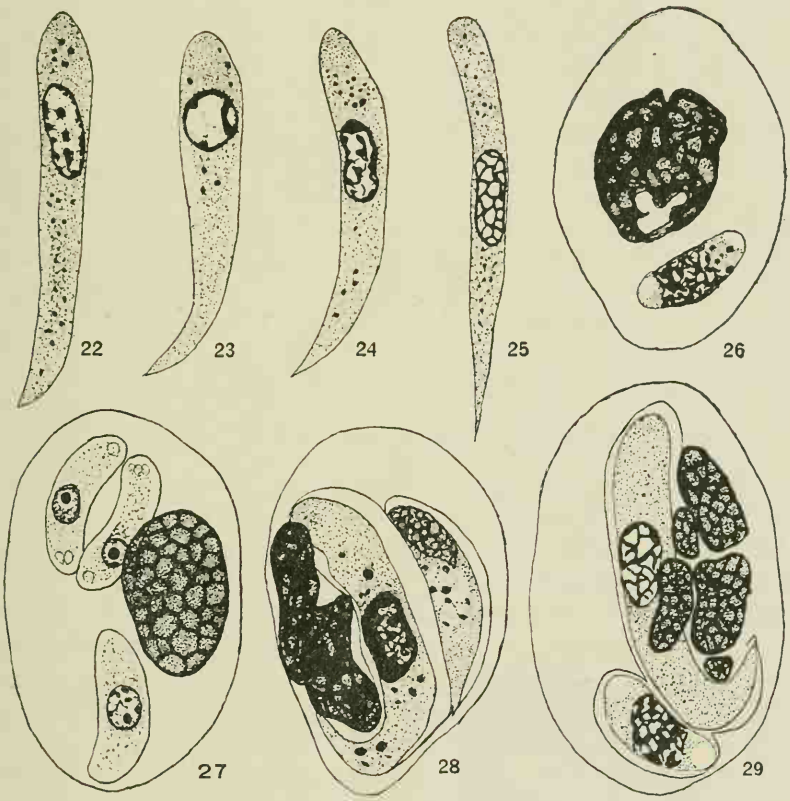
somewhat. The former is shorter and thicker and its anterior end is less truncate with a nucleus situated near the anterior extremity, while the latter is longer and thinner and its anterior end is more truncate with a centrally located nucleus. I have not noticed the difference in living condition, as their length and shape changed from time to time due to the movements. The difference noted in the stained preparations may indicate different circumstances in preparing them, although practically same methods were used in both cases.



Figs. 14 to 21. *Haemogregarina* sp. Fig. 14, a normal red blood corpuscle. Fig. 15, a trinucleate erythrocyte containing an intracellular stage (the structure of one of the nuclei is omitted). Figs. 16 to 18, three infected erythrocytes. Fig. 19, an erythrocyte containing two parasites. Fig. 20, the parasite is just leaving the host cell. Fig. 21, an extracellular giant form still covered with an envelope. Schaudinn: Delafield. x 2100.

The free haemogregarines may be seen usually soon after the preparation was made, but they increase in number in a few minutes. I have often noticed the fact that when a fresh preparation of a small portion of the lung of an infected frog was made, the number of extracorpuscular forms

increased from 1 to 2 to from 12 to 20 in each field (compensation ocular 4 and apochromatic objective 8 mm.) in five minutes while under observation. The body is rounded or truncate at the anterior extremity and tapers to an attenuated posterior end. As was noted by many authors in several species of haemogregarines, the posterior end of the animal is seen connected with a thread-like structure sometimes measuring twice as long as the body. It seems to me that it is a portion of the cytoplasm of the host cell which was in direct contact with the parasite before the latter left it,



Figs. 22 to 29. *Haemogregarina* sp. Figs. 22 to 24, extracellular stages from the lung capillaries of *Rana pipiens*. Fig. 25, an extracellular form from *Rana clamitans*. Figs. 26 and 27, erythrocytes of *Rana pipiens* with small forms. Figs. 28 and 29, erythrocytes with an individual of *Haemogregarina* sp. and a smaller form. Fig. 24, Giemsa; Fig. 27, Heidenhain; the rest Delafield. x 2100.

and which became left behind as the animal moved forward. This view also seems to be reasonable if one considers the fact that the thread-like connection is most conspicuous soon after the parasite leaves the host cell and disappears in a few minutes. The structure of the body in

stained state is similar to that of the intracellular stage. The nucleus assumes sometimes ring form. The average dimensions of forms found in *Rana pipiens:* length 23.8 μ, largest breadth 3.6 μ: and of those found in *Rana clamitans:* length 27.6μ, largest breadth 2.4μ.

Development.—Ordinarily the dimensions of the parasites of both phases described above are somewhat uniform. No stages of division were noted either in the host cell or in free state. Smaller intracellular stages such as shown in Figs. 26 to 29, are often observed. They seem to occur always in the host cells. Oval with a flat or concave and a convex side, they show at each end of the body one to three vacuole-like structures in both fresh and stained conditions. These forms are always present within the same host animal with the large forms described above. But no intermediate stages between them have been seen, although a few forms such as shown in Figs. 28 and 29 are noticed. Without infection experiment, I cannot say whether they are only different stages in the development of one and the same parasite or entirely different forms. Stebbins (1904) considered the smaller form as a distinct species and named it *Haemogregarina catesbianae.* The development of haemogregarines of frogs has not yet been worked out, although *Haemogregarina stepanowi* found an earnest worker in Reichenow (1910) who described interesting observations.

IV *Trypanosoma rotatorium* (Mayer)

Synonyms.—*Paramoecium loricatum* Mayer 1843, *Paramoecium costatum* Mayer 1843, *Amoeba rotatoria* Mayer 1843, *Trypanosoma sanguinis* Gruby 1843, *Monas rotatoria* Lieberkühn 1870, *Undulina ranarum* Lankester 1871, *Paramoecioides costatus* Grassi 1882.

Habitat.—In the blood of *Rana esculenta, R. temporaria, R. clamitans, R. pipiens, R. castebiana, R. galamensis, R. oxyrhynchus, R. mascarensis, Rappia marmorata, Bufo vulgaris, Bufo regularia, Letodactylus ocellatus, Hyla viridis and H. arborea.* A number of host frogs whose specific names were not determined by the original authors are excluded.

The trypanosomes are more numerous in the blood vessels of organs such as liver and especially kidney than in the peripheral or heart blood.

Historical.—Since Gluge (1842) found the organism, several workers noted and studied the flagellate, the chronological review of which is found in Laveran and Mesnil (translated by Nabarro, 1907). Doflein (1910), Lebedeff (1910) and Machado (1911) are more recent conributors to our knowledge concerning this blood parasite of frogs.

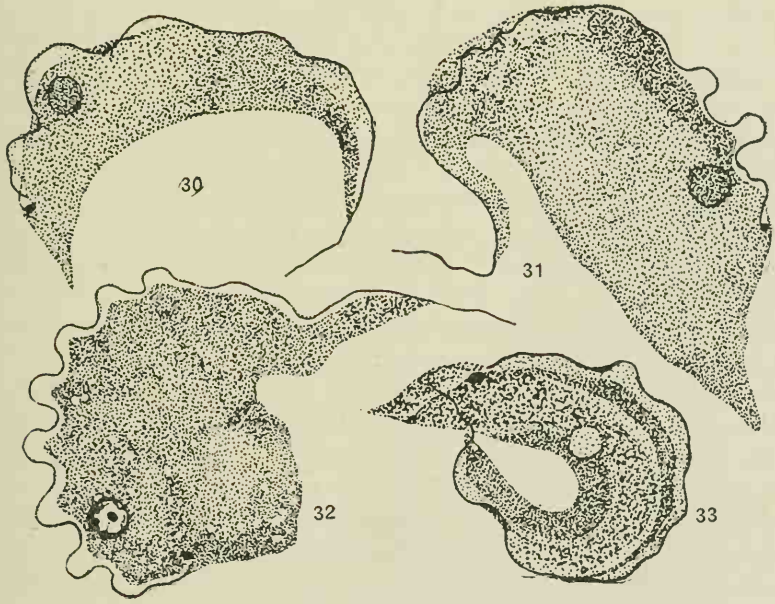
Distribution.—Europe, Africa, Asia, South and North America.

Methods of observations.—The blood should be examined as soon as it is taken from the frog. With a capillary pipette, draw the blood from the frog heart. If it is taken aseptically, the blood can be kept in sterile

condition in test tube with physiological solution and the trypanosomes will live for several days. If one cannot observe the preparation soon after the blood was taken, make temporary hanging drop preparations which may be examined in two to six hours. The trypanosomes are the largest ones known up to date, and its presence can be detected under a low power, although one may have to examine several preparations of the frog blood before finding one individual. When alive one can distinctly see the undulating membrane and the characteristic wriggling movement of the trypanosomes.

To make permanent smears, make ordinary blood smears and fix with either sublimate-alcohol-acetic mixture or with absolute alcohol (for 10 to 20 minutes). Staining with Delafield's haematoxylin, Heidenhain's iron haematoxylin or Giemsa's stain, will bring out morphological details. The nucleus is sometimes hard to stain and prolonged staining is needed to demonstrate its structure.

Morphology.—Polymorphic. Earlier observers held that the difference in size and form among different individuals showed that of the specific characters which view has however been abandoned by modern investiga-



Figs. 30 to 33. *Trypanosoma rotatorium*. Fig. 32, Delafield; rest Giemsa. x 1500.

tors. When the blood is examined soon after its removal from the frog heart, one will see broad forms as well as slender ones mingled with intermediate forms. After some time, some individuals become rounded. The majority have more or less attenuated extremities. The form of the

body changes constantly with the striking movements of the undulating membrane. The flagellum which runs along the outer margin of the undulating membrane is very frequently seen to extend beyond the anterior end of the body. Its length varies, and may not be seen at all. The blepharoplast and nucleus can hardly be seen in actively motile individuals. The cytoplasm is granulated, may contain rounded spaces especially near the posterior margin, and shows in many individuals longitudinal striae.

When stained, the small oval or oblong blepharoplast is seen located some distance from the posterior tip of the body. The flagellum seems to take its origin a short distance from the blepharoplast. The nucleus is located near the blepharoplast and on the same side of the body where the latter is situated. It is rounded and shows its structure poorly with any stain. It contains chromatin granules collected along the periphery. A small karysome may sometimes be seen in the central region. The cytoplasm shows numerous small vacuoles in the posterior half of the body. Dimensions vary considerably. Length, 44 to 70μ and breadth 10 to 35 μ.

Development.—Although artificial cultivation in vitro of *Trypanosoma rotatorium* has successfully been carried out by Bouet, Doflein, Lebedeff and Machado, we know practically nothing concerning its development in the frog body. Some authors such as Laveran and Mesnil, Doflein, etc., have not seen trypanosomes undergoing division in the blood of host frog. My own examination of numerous preparations also leads me to agree with them on this point. On the other hand, França and Athias (1906), Dutton, Todd and Tobey (1907) and Machado (1911) observed stages in division in the smears of frog blood or in preparations of fresh blood of infected frogs "kept aseptically at 72° to 89° F. for two or three days" (Dutton, Todd and Tobey).

According to the observations of the last named three investigators, the trypanosome after losing its flagellum, became rounded and underwent active division, producing numerous small rounded organisms—"forty-one cells were counted, though they were probably more." Each body became ovoid, then pear-shaped, and from the more rounded end a flagellum was produced. These young forms became active and free from the outer covering of the original trypanosome. They divided rapidly by splitting longitudinally increasing in number. They were herpetomonas-like and remained in this condition until the preparations were discarded. The authors studied further stages in stained smears, and stated that the herpetomonas-like forms developed into *inopinatum*-like forms which were also "found in fresh blood, in contradiction to the forms just described, which were found in kept blood alone."

Machado describes stages in division of the trypanosomes in frog. From his statement, it is not clear whether he found these stages in the

blood or kidney. Figs. 14 to 19 and 36 to 40 given by Machado are rather isolated from others and hard to be reasonably connected with the other stages which he figured. I have not had chance of examining *Leptodactylus ocellatus* myself, but comparison of the above mentioned Machado's figures with the vegetative stages of a Myxosporidian, *Leptotheca ohlmacheri* which is described very briefly in this paper and in details in the other paper (Kudo, 1922) and which is not uncommon parasite of the kidneys of *Rana clamitans*, *R. pipiens* and *Bufo lentiginosus* of the United States, leads me to think that the frogs studied by Machado were probably infected by the Myxosporidian or an allied form besides the trypanosomes. Machado states that trypanosomes were abundantly seen in the kidney of the host which fact I also noted. He seems to have mixed the stages of development of a Myxosporidian with those of the trypanosomes.

Judging from the trypanosomes of fishes and reptiles, and *Trypanosoma inopinatum*, another member of the genus parasitic in *Rana esculenta* of Algeria, the present species seems to undergo changes in the body of blood sucking invertebrates. Fuller accounts of the life history of the trypanosome awaits future investigations.

V *Trypanosoma parvum* nov. spec.

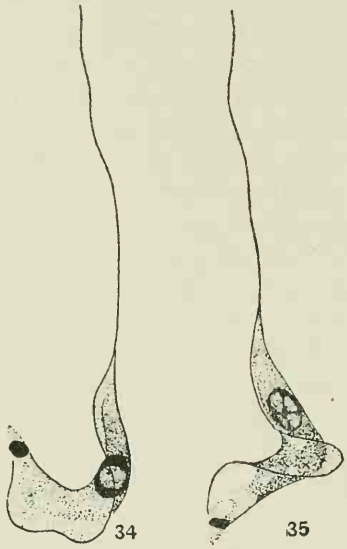
Habitat.—In the blood of *Rana clamitans*. Fourteen specimens were examined between July and September, 1920. In one of them a fairly heavy infection of a trypanosome was noticed. Five to eight active individuals were recognized in every field (compensation ocular 4 and apochromatic objective 8 mm.). The frog also harbored *Trypanosoma rotatorium* in small number (one individual in every two other field under the same combination as noted above), but no haemogregarine was found. I have not seen it since that time, although I have examined about four dozens of *Rana pipens* which were purchased from a Chicago biological supply store.

Methods of observations.—Same as *Trypanosoma rotatorium*. For demonstration the unusually long flagellum, Fontana's staining was used with satisfactory result.

Morphology.—When alive, the movements can be distinguished into two types: travelling and wriggling movements, of which the first is prominent. The active wriggling movements remind one of those of *Trypanosoma lewisi*. The undulating membrane is fairly well developed. The nucleus and belpharoplast are faintly visible, while the relatively long flagellum can distinctly be seen with an oil immersion objective. The cytoplasm contains frequently small rounded clear spaces, and is more or less vacuolated at the posterior portion.

When stained, one finds in them structures typical to a trypanosome. The body is spindle-shaped usually being curved in an arch or S. The

posterior end is ordinarily attenuated and ends in a blunt point, while the anterior extremity is more sharply pointed. The cytoplasm is usually dense from the anterior part to the middle region of the body, while a clear area is frequently seen just posterior to the nucleus, either close to or



Figs. 34 and 35. *Trypanosoma parvum* nov. spec. Fig. 34, Giemsa; Fig. 35, Delafield. x 3300.

somewhat separated from it. The posterior portion is more or less vacuolated as was noted in living specimens. The blepharoplast is located very close to the posterior tip of the body. It is relatively large, and rounded or oblong in shape. The flagellum that borders the outer margin of the undulating membrane does not seem to take its origin directly in the blepharoplast, but arises from a point inconspicuously marked at some distance from the latter. The free portion of the flagellum reaches 15 μ in length, though its length varies most widely. The nucleus is rather large, and is located between the middle and anterior third of the body. It is spherical or oval. In Giemsa stained smears, the peripheral portion stains very deeply, while the central portion is occupied by a few linin threads. A karyosome may sometimes be seen eccentrically located.

Dividing forms were not seen. The trypanosomes are strikingly uniform in size, showing little variation in size and general shape, except the length of the flagellum. Measurements of two hundred specimens in smears fixed with sublimate-alcohol-acetic mixture and stained with Giemsa's solution are as follows: length of body, exclusive the flagellum, 11 to 14 μ, largest breadth including the undulating membrane, 1.2 to 1.9μ, length of free portion of the flagellum 5 to 15 μ.

Of all the trypanosomes of amphibians known up to date *Trypanosoma inopinatum* Sergent et Sergent, 1904, resembles closely to the form just stated. These two forms resemble each other in the dimensions and general resemblance to *Trypanosoma lewisi.* There are however some differences in the location of blepharoplast, the structure of cytoplasm and the general form of the stained individuals which shows the activity of the two forms is not same. The blepharoplast is located more closely to the posterior tip in this form than in Algerian form. The breadth of the American form is 1.2 to 1.9 μ, while that of *Trypanosoma inopinatum* is 3 μ. The cytoplasm of the present form is vacuolated at the posterior portion of the body, while the Algerian form, according to Sergent and Sergent's figures, is uniformly granulated. Furthermore the activity of the two forms appears to be quite different. In the forms I have studied the body shows an arch or S shape in stained smears, while Sergent and Sergent figure more or less straight form, thus indicating probable difference in their activity when alive. Consequently these two forms should better be separated from each other by different specific names, until I am able to compare the preparations of them.

Since the cultivation of *Trypanosoma rotatorium* in vitro has been attempted by Lewis and Williams (1905), the fact that the trypapnosomes undergo division in the culture media resulting in the formation of small spindle-shaped bodies resembling in appearance to Herpetomonas or Crithidia, became known. But in no case, a structure typical to a trypanosome was noted among these small forms. At our present state of knowledge concerning trypanosomes, it is proper for us to consider the extremely small trypanosome described above as independent from *Trypanosoma rotatorium.* As it is morphologically distinguishable from a closely allied form, *Trypanosoma inopinatum,* I propose to name it provisionally *Trypanosoma parvum* nov. spec.

Parasitic flagellates in the intestine

Number of parasitic flagellates have been described in the intestine of frogs. The reader is referred to Dobell (1909) and Swezy (1915, 1915a) concerning them.

VI *Opalina* sp.

The Opalinas described here seem to be identical with *Opalina ranarum* Purkinje et Valentin 1835.

Habitat.—In the rectum of *Rana clamitans* and *R. pipiens.*

Historical.—A complete chronological review of works on Opalinas will be found in Metcalf (1909).

Methods of observations.—The rectum of frog is placed in a small watch glass and opened in physiological salt solution under dissecting microscope. When the preparation is made, the Protozoon will be seen actively moving. In order to retard the active movements, a drop of

two of cherry gum solution may be added. The ciliary movements and the structure of the body can easily be studied. For permanent preparations, follow the methods stated for *Entamoeba ranarum*.

Morphology.—The body is broadly oval, with blunt anterior and more rounded posterior extremities. One side is convex, while the opposite side exhibits a shallow depression at the middle part. The body is highly flattened. Parallel rows of cilia run obliquely. The body is covered with cilia of uniform length. The protoplasm is sharply differentiated into

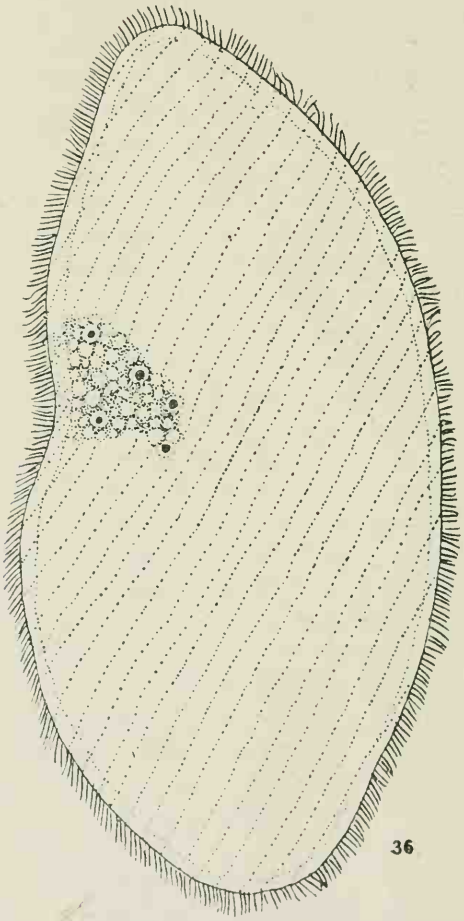


Fig. 36. *Opalina* sp. Delafield. A portion of the body is shown in detail. x 400.

ectoplasm and endoplasm. The ectoplasm is hyaline near the pellicle, but is alveolated near the endoplasm. The latter is granulated in living individuals, but when stained with Delafield's haematoxylin, it shows a vacuolation. The endoplasm contains a large number of nuclei of uniform size. Cytostome or cytpyge is not observed. Dimensions: length 130

to 200 μ, breadth 50 to 120 μ. Occasionally large form reaches 500 μ in length.

Development.—The Protozoon divides in the intestine of the frog, stages of which are commonly seen in the rectum in the summer. I have not studied a new infection of a host frog. According to Neresheimer (1907), *Opalina ranarum* divides successively in the rectum of host frog in the spring, and produces numerous small individuals, each containing a few nuclei. They encyst by producing a hyaline and resistant membrane around them. The cysts come out of the host body with fecal matters and remain on the bottom of the water. When the young tadpoles swallow the cysts, the contents of the latter leave the membrane in the rectum of the new host. The free young opalinas become differentiated into gametes by division and after fusion form zygotes. The zygotes grow into adult ones as the tadpoles metamorphose themselves into adult frogs.

Summary

1) The main object of the present paper is to furnish a brief account of Protozoa parasitic in common North American frogs for general students in Zoology.

2) The occurrence of *Entamoeba ranarum* in *Rana clamitans* is stated.

3) A Myxosporidian, *Leptotheca ohlmacheri* is studied in the kidneys of *Rana clamitans* and *R. pipiens*.

4) *Trypanosoma rotatorium* of *Rana clamitans* and *R. pipiens* is studied.

5) *Haemogregarina* sp. in *Rana clamitans* and *R. pipiens* is studied.

6) A new trypanosome, *Trypanosoma parvum* is described from *Rana clamitans*.

7) *Opalina* sp. from *Rana clamitans* and *R. pipiens* is studied.

8) Methods of observation and brief review of previous works for each of these forms are given.

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A NEW SUCTORIAN FROM WOODS HOLE

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The aberrant group of the Infusoria known as the Suctoria have been but little studied in the United States. In Europe, however, the monographic work of Sand and, more recently, of Collin have made them well known.

In 1914 I published some notes on the reproduction and food reactions of a fresh-water species of the genus Podophrya which appeared in hay-infusions at Baltimore. Through this work I became interested in this peculiar group of the protozoa and during the summers of 1916 and 1917, while at Woods Hole, Mass., I collected and studied five species of Suctoria which attached themselves to the stalks of the common hydroids, Obelia commissuralis and Obelia geniculata.

The four previously described species found were as follows:

Ephelota coronata (Wright).

Acineta tuberosa Ehrenberg.

Paracineta livadiana (Mereschowsky)—This is probably the species referred to by Calkins (1901) as "Acineta divisa Fraipont."

Ophryodendron abe itinum Claparede et Lachmann.

Besides these four species another was present which was obviously new and extremely remarkable, showing decided resemblances to Acinetopsis rara Robin.

The genus Acinetopsis was established by Robin (1879) to contain a single species, Acinetopsis rara, characterized by the presence of a stalked theca with free apical margin (a "coque" in the terminology of Collin [1912 page 117] and by the presence of a single extensile, flexible tentacle in the center of the apical surface of the body. This species has never been reported by any other observer. Martin (1909) attempted to identify Robin's Acinetopsis rara with one stage in the life history of his new species, Tachyblaston ephelotensis. This identification has not been accepted by Collin in his monograph of the Suctoria, and will hardly be agreed to by anyone who will take the trouble to compare Martin's figures with the original figure of Robin, as reprinted in the Journal of the Royal Microscopical Society (1880). More recently Collin (1909, 1912) has described a new species under the name of Acinetopsis campanuliformis. This species is described as having a bell-shaped "loge" or closed theca,

and bears six flexible tentacles. Collin seems to have overlooked the statement of Robin that Acinetopsis rara has a theca with free margin, and has classified Acinetopsis as a genus with closed theca. If this difference be really of generic importance, Collin's Acinetopsis campanuliformis must be placed in a new genus.

The species which I found at Woods Hole so closely resembles Acinetopsis rara at one stage of its life-history that I am not willing to erect a new genus for it, in spite of the fact that in this new species I find that the flexible extensile organ proves to be a seizing organ, analogous to the pointed tentacles of Ephelota and without any suctorial function. To this new species I have given the name of "tentaculata," because in its adult condition it is provided with true sucking tentacles as well as with the elongated "probosces" which I consider characteristic of the genus.

Description of Acinetopsis tentaculata n. sp.

Protoplasmic body enclosed in a flattened, cup-shaped theca with a free apical margin, borne on a slender stalk a little longer than the theca itself. (See Figure 1). Body irregularly flattened-ovoid in shape, bearing on the apical face one or two probosces. Each proboscis is a very mobile organ, which can be bent in any direction, extended until it is a mere thread twice the length of all the rest of the organism, or retracted until it is less than half the length of the protoplasmic body alone. Structurally, the proboscis consists of a homogeneous central strand about whose outer surface is wound spirally a ribbon of protoplasm evidently contractile in nature. Each proboscis is tipped with a large, highly refractive globule of adhesive and viscous character. That the probosces are firmly anchored to the body is self-evident from the observations given later regarding their use in feeding. As is shown in Figure 2, this attachment is well below the surface of the body, but I have not been able to make out the details. Besides these probosces the apical face of the body also bears about twenty or thirty short stout tentacles of the familiar capitate type, these being distributed in two groups, one on either side of the insertion of the probosces.

The macronucleus has irregular outlines, but is in general roughly ovoid. One or more small spherical micronuclei are present. A single contractile vacuole is located near the base of the body.

The measurements of a typical mature specimen were as follows:
Protoplasmic body—138 microns long, 100 microns wide, 73 microns thick.
Theca—187 microns long, 105 microns wide.
Stalk—287 microns long.
Proboscis when fully extended—about 500 microns long.

Feeding Habits

As far as I have been able to observe, Acinetopsis tentaculata feeds only on Ephelota coronata, but it attacks this common species with voracity. The probosces are extended and moved about until the globule at the tip of one of them comes in contact with the body of an Ephelota. It seems to secure a firm attachment at once and in a few cases where the Ephelota was very firmly attached to its stalk I have seen the globule elongate and finally pull in two rather than release its victim. As soon as the attachment is secured the proboscis contracts strongly, drawing the body of the Ephelota within reach of the short sucking tentacles of its captor. Sometimes the stem of the Ephelota is long enough so that this can be accomplished by merely bending it. More often the attachment between the body of the Ephelota and its stem must be broken by a rapidly repeated series of jerks due to sudden contractions of the proboscis. When the Ephelota finally comes within reach it is firmly seized by the sucking tentacles and its internal protoplasm sucked out at leisure. One of the difficulties in the study of this species was the long search which was always necessary to find an individual whose body was not almost entirely concealed by several Ephelotas in process of being sucked dry.

Reproduction

The actual escape of the embryo was not observed, but Figure 3 shows plainly that reproduction occurs by the same process of simple (i. e. not multiple) internal budding which is characteristic of all the genera of the family Acinetidae. Nor have I observed the entire series of stages in the attachment and growth of the free-swimming ciliated embryo. From the young individuals I have found in nature, it seems probable that after attachment and the formation of a stalk and theca, a single proboscis is first formed (Figure 4). This condition is structurally a perfect duplicate of Robin's Acinetopsis rara. Slightly larger individuals still show only a single proboscis but have also formed a number of true tentacles (Figure 5). And finally, in mature individuals, we find the two probosces characteristic of the species.

There is a great temptation here to suggest that these growth stages may reflect something of the course of evolution in this genus. It seems quite probable, for example, that the second proboscis, the last organ acquired by the individual, is a recent evolutionary acquisition of the species. It would probably be going too far to intimate that the stage with a single proboscis and no tentacles harks back to some ancestor in which the proboscis was a true sucking tentacle and no other organs for capturing prey were necessary. However, this must be the condition which obtained in Robin's Acinetopsis rara, unless he overlooked the inconspicuous tentacles or was dealing only with a growth stage.

Summary

Acinetopsis tentaculata n. sp. is described and figured. It is characterized by having a stalked "coque" or theca with free apical margin, and by the presence of one or two extensile seizing organs or probosces as well as suctorial tentacles. It feeds on Ephelota, seizing them and drawing them within reach of its tentacles by means of the probosces. It reproduces by internal budding, forming free-swimming ciliated embryos which settle down and gradually grow into the adult form.

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Description of Plate

All figures are camera drawings from specimens killed in Dubosque's alcoholic modification of Bouin's Fluid and stained with picric acid haematoxylin.

Figure 1. Acinetopsis tentaculata. Side view of entire mature specimen with one proboscis partly extended and the other nearly retracted. x 185.

Figure 2. Acinetopsis tentaculata. Front view of body and theca of mature specimen. Note deep attachment of probosces and arrangement of tentacles in two groups. x 350.

Figure 3. Acinetopsis tentaculata. Front view of an individual in which the macronucleus is just dividing to form the macronucleus of an internal bud. x 350.

Figure 4. Acinetopsis tentaculata. Young form with a single proboscis and no tentacles. x 350.

Figure 5. Acinetopsis tentaculata. Young form with a single proboscis and a few tentacles. x 350.

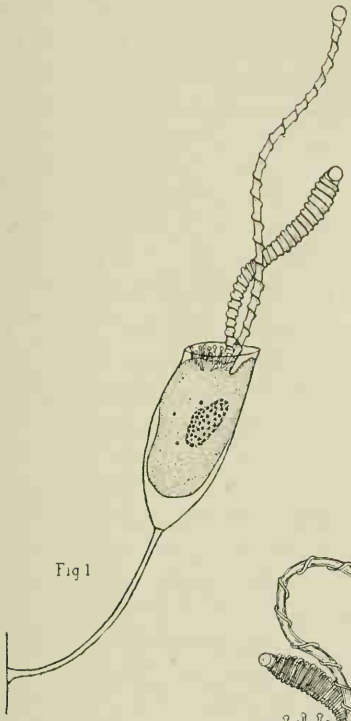

Fig 1

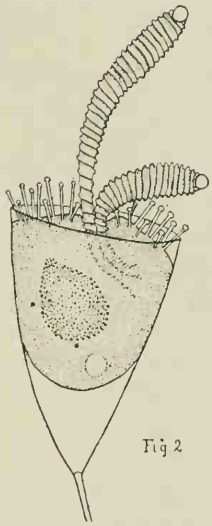

Fig 2

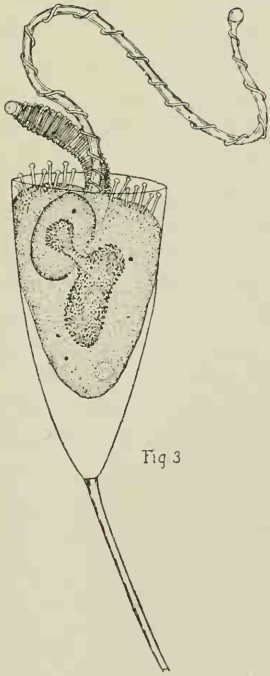

Fig 3

Fig 4

Fig 5

DEPARTMENT OF SUMMARIES
DEVOTED TO DIGESTS OF PROGRESS IN BIOLOGY

TEN YEARS OF HEREDITY[1]

By
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Though no one is likely to be misled by my subject into supposing that the laws of heredity have been in operation only a decade, it may not be universally appreciated that heredity is one of the oldest of biological phenomena. It is at least as old as, probably older than, organic evolution of which we have long been accustomed to speak in terms of millions of years. For, when the first living thing, if ever there was such a being, gave rise to a second, by reproduction, this second living thing was either like its parent, or different from it. If like its parent, heredity had begun. If different from its parent, it was almost certainly different in only one or a few respects, but like the parent in the rest, in which case both heredity and evolution were in operation.

The stipulated ten years to which my title refers are not, however, an arbitrary limit set for the purpose of relieving me of the necessity of covering the whole of a very large subject. They are a period which, in the development of knowledge of heredity, is naturally marked off from the numerous decades that precede. Those of you who possess a little knowledge of heredity, to whom the name of Gregor Mendel has a fascinatingly familiar sound, and in whose memory lingers the date of 1900 in which year the famous Austrian monk's long hidden experiments were again brought to light, may wonder why I should wish to describe the developments of but the latter half of the period since that rediscovery. It is true that the great interest aroused by the verification of Mendel's Law, with the multiplication of experimental work which was induced by it, was a necessary precursor of the events with which I propose to deal. But about 1910 there began a chain of discoveries, which have followed one another in unbroken series to the present time, and which have led to a conception of the operations of heredity of a degree of complexity, and withal of harmony, which even the most sanguine twenty years ago would not have ventured to predict.

[1] This lecture, delivered before the Graduate Club of the University of Michigan, was designed to present to persons without biological training, not a résumé of all important work in heredity in the period referred to, but the point of farthest advance and the principal work leading to it.

Former Lack of Analysis

Heredity had long been discussed in terms of averages. In popular discourse it was always so, children were replicas of their mothers, or were chips out of the old block, or the son of a Cholmondeley was the image of a Jones. The *ensemble* of characters was considered. Even those who were professionally engaged in the study of heredity lumped together many things now known to be partially or wholly distinct from one another, regarding them as a single trait. Stature, obviously made up of many elements, was treated as a single characteristic. Intelligence, likewise compound, was studied as if simple.

There was not wanting, it is true, even among the laity, an analytic tendency. A youth would have his father's mouth, his mother's eyes, his grandfather's complexion. But it was not until the emergence of Mendel's work in 1900, and the multiplication of investigations consequent upon that event, *that it was realized to what extent inherited traits may be separated from one another as distinct and independent units.* Eyes were inherited independently of hair, hair color independently of hair form, color independently of distribution of color, whether uniform or in patches. Unit characters became distinctly vogue. Anyone who could utter the magic expression "unit characters" and speak the name of Mendel with his first name and title, had thereby established his right to be regarded as a thoroughly modern geneticist.

Difficulties of the New Conception

All this development raised in the biological mind certain difficulties. When it was inquired how all these unit characters were manipulated independently of one another, there were obstacles—that is, when the offered explanation passed a certain point. To speak intelligibly of these difficulties it will be necessary to refer briefly to a few elementary facts of biology.

All organisms are composed of units of structure called cells. These cells regularly contain, as part of their structure, a rounded body, the nucleus, which stains deeply in most dyes and which is therefore conspicuous in most common microscopic preparations. The size of an organism is increased usually by the multiplication of cells, which is accomplished by the division of the cells already present. In the process of division, the cells develop a complicated figure in which the highly staining material of the nucleus is resolved into a number of distinct bodies called chromosomes. As the division is completed, the chromosomes lose their distinct form, producing a nucleus in which separate bodies are no longer visible; but at the next division the chromosomes appear again, in the same number as in the previous division. This number is in general constant in all cells of the same individual, and, barring some differences between the

sexes, is constant for all members of the same species. Moreover, in animals in which the chromosomes are not all of the same size or shape, *each dividing cell reveals the same number of chromosomes of each shape or size.*

Since all organisms are composed of cells, the phenomena of heredity must in some way be traceable to cells. The constancy of occurrence of the nucleus, and of a given number of chromosomes in the nucleus, early gave rise to a suspicion which later, on a foundation of fact, ripened into a conviction, that in these structures is the mechanism through which heredity is governed. If it were assumed that the factors of heredity were contained in the chromosomes, many things would be explained. Reference will be made now to only one of these things.

One of the new features of discussion of heredity was the attention devoted to unit characters. How were these characters operated as units, independently of one another? Chromosomes provided the answer. It must be understood that in all the higher animals and plants, *no parent contributes all of its chromosomes to any one offspring, but only half of these chromosomes.* In the development of the germ cells a peculiar cell division called the reduction division takes place in which *the chromosomes separate into two groups, one group being enclosed in the one daughter cell, the other group in the other cell.*

Chromosomes and Recombination of Characters

In the composition of these groups of chromosomes, there is a wide range of different possibilities. In some cases, the chromosomes may be

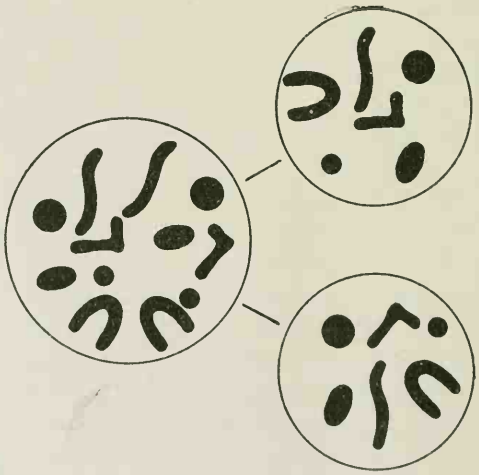

Figure 1. Diagram of a cell in which the chromosomes are capable of arrangement in pairs, the two chromosomes of each pair being precisely alike in all respects. Such a cell, in maturation, divides into two cells, each with half the number of chromosomes, and each exactly like the other in all hereditary factors. The shapes of the chromosomes are not actual, but only a diagrammatic representation of the likenesses and differences in their hereditary composition.

capable of assortment in pairs, as in figure 1; that is, there are two chromosomes in each cell that are exactly alike, two other chromosomes that are exactly alike but different from the first pair, and so on. In such a case, by separating the members of each pair, two groups of chromosomes may be made up which are identical. In such a case, therefore, *two germ cells with exactly the same hereditary possibilities are produced*, and the parent may contribute precisely the same hereditary traits to every one of its progeny. Moreover, it can transmit to each of its offspring every hereditary trait of which it is possessed.

In other individuals, on the contrary, every chromosome of a cell may differ in one or more respects from every other chromosome, as in figure 2.

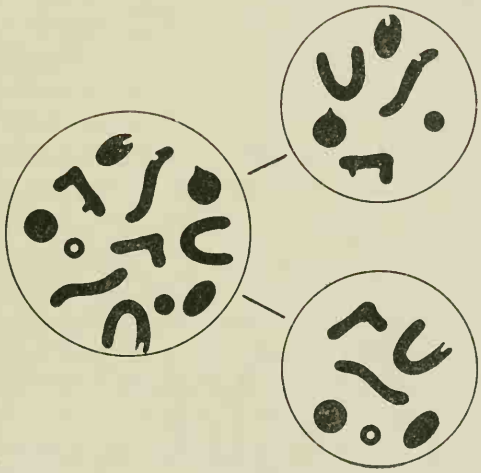

Figure 2. Diagram of a cell in which the chromosomes may be arranged in pairs, but the chromosomes of one pair are not exactly alike. The chromosomes of a pair may be alike in most respects, but different in one or more features. Such a cell, in maturation, divides into two cells which are alike in the main but differ in certain hereditary factors. The precise hereditary composition of each cell therefore depends on how the chromosomes are distributed to the two cells.

In such a case, in the reduction division at which only half the chromosomes are conveyed to each daughter cell, it is not possible to produce two cells that are identical. *Each chromosome in each of these two cells is different from every chromosome in the other cell.* Moreover, the parent is here contributing, with respect to certain characteristics, only half of its hereditary potentialities to any one of its offspring.

Between these extremes, in which, on the one hand, the parent hands on all its hereditary traits to all of its offspring, and, on the other hand, transmits only half of its possibilities with respect to certain features to any one offspring, there are all intermediate grades. The result depends

on how the chromosomes are separated into two groups. In that cell division in which each daughter cell receives only half the total number of chromosomes, it appears to be a matter of chance, subject to certain restrictions, how the half number shall be made up. If I have not made this procedure clear, the following analogy will be useful. If it is proposed to divide by chance a group of buttons, or poker chips, if that be a more familiar figure, of a variety of colors, into two groups of six each, it is obvious that the groups of six may be very unlike; also, that if the same dozen buttons be divided into two groups again, the second division may be very unlike the first. If these buttons represent chromosomes, and their colors stand for hereditary traits, it is clear that these traits may be distributed in very different ways to different offspring.

The chromosomes, then, because they act to some extent independently of one another, *offer an explanation of the independence of unit characters*—provided only that the things which produce these characters are in the chromosomes. There are other reasons, equally good, perhaps better, for believing that the hereditary factors, as they are called, are in the chromosomes, but this additional evidence may pass.

Individuality of the Chromosomes

All this conception of the operations of heredity, in relation to the chromosomes, was arrived at before the ten year period of which I am eventually to speak. But certain difficulties are inherent in the conception. The number of chromosomes in the cells of an animal is strictly limited. In man, one author fixes the number at 48, another at 24. In other animals there is better agreement, and the number is as low as four, or even two. In man, even the largest number suggested, 48, must be much smaller than the number of traits which he inherits. If this be true, *the representatives of several traits must reside in the same chromosome.*

The difficulty involved in this situation was that *the chromosomes were believed to be individuals.* That is, the chromosomes which become distinguishable at one cell division were held to be the same identical chromosomes, part for part, as were observable at the preceding cell division; and chromosomes occurring in one individual were believed to be identical with those of its parents. There were many facts concerning the shapes of the chromosomes, and their behavior at various times, which lent support to the view that they are persistent individual objects.

Were this regularly true, *two hereditary traits represented by something in the same chromosome would necessarily behave as a single characteristic.* They could not be independently assorted, when the chromosomes were separated into two groups in the reduction division in the production of germ cells, but would go together. Traits represented in different chromosomes would be independently assorted, but those in a single chromosome would act as a unit.

Requirements of Proof of Linkage

Whether this condition actually existed in any animal or plant was for a long time not known. *To determine whether inherited traits were ever bound together in groups required an animal in which differences in a considerable number of characteristics existed in different individuals.* It required also a careful study of such an animal or plant to determine whether the traits were wholly independent, or were grouped. Early in the revival of Mendelism, an association of certain hereditary traits with sex was demonstrated, but indications of an association of hereditary traits with one another were long delayed. The number of traits whose inheritance was understood, in any one species, was too small.

Then came the year 1910. In that year a fly was born— or hatched. It belonged to the small brownish gray species which is seen every summer day hovering about fruit stands or garbage pails. This species had been bred for years in a number of laboratories, notably those of Columbia University by Professor T. H. Morgan and his students. Then one afternoon, in a bottle, appeared the fly of which I speak, which differed from all others in the bottle, and from all of its ancestors for many generations, in having white eyes. Flies of this species regularly have red eyes. Since 1910, other eye colors have appeared, vermilion, cherry, eosin, buff, tinged, blood and purple being the names applied to some of them. Other flies were produced which had unusual wings—short, blunt, crumpled, or missing, curled up, curved down like an inverted bowl of a spoon, or spread at an angle. Other parts of the body likewise presented variations. The spines became forked, or reduced in number. Extra legs were produced. The color of the body became yellow or black in certain individuals. Physiological changes not producing any observable structural differences have also been detected. All told, over two hundred such modifications have been discovered in this one species of fly since 1910. Most of these characteristics were found to be definitely inherited. Fortunately, many of these altered flies were quite healthy, were easily reared, and have been carefully studied. *The fruitfly was obviously the organism by which the individuality of the chromosomes and their relation to heredity could be tested.*

Early Demonstrations of Linkage

This test came gradually. It was found that these characteristics were not wholly independent of one another. Thus, white eye and yellow body-color were very closely associated with one another. When a white-eyed and yellow-bodied fly was crossed with a normal red-eyed and gray-bodied fly, their offspring in certain subsequent generations, in certain cases should have shown all combinations of the two eye colors and the two body colors with equal frequency. That is, if the four traits were inherited independently of one another, white eye and gray body

should have been combined in one individual as often as white eye and yellow body. But they were not; white eyes and gray bodies were found together in only about one-fiftieth as many cases as would have been expected. White eye was nearly always associated with yellow body in these crosses.

It was discovered, also, that white eye color was associated in the same way, though less closely, with sable body color, with club shaped wings, and a number of other characteristics. Moreover, if white eye color was thus bound up with a certain characteristic, yellow body color was also associated with the same characteristic. And all characteristics that were thus associated with white eye and yellow body were found to be linked—that is the word Morgan uses—with one another. *All these traits behave, to some extent, as a unit. They are not absolutely bound together, but they hang together more frequently than the chance assortment of chromosomes, or colored buttons, or poker chips, would lead one to expect.*

Independent Linkage Groups

Approximately forty of the more than two hundred new characteristics that have arisen in this fly in the past ten years may safely be said to belong to the group that is linked with white eye color and yellow body. Long before all of these had been discovered—indeed, when only a few of them were known—certain other new traits had come into existence which were definitely *not* linked with white eye. One of these was a short crumpled wing which has been called vestigial. In crosses which involve vestigial wing and white eye at the same time, the occurrence of vestigial wing in the individuals of subsequent generations bears no relation to the occurrence of white eyes in the same individuals. The chances are even, in such crosses, that a fly with a vestigial wing will have white eyes in as large a proportion of cases as will a fly with normal wings. Likewise, there is no relation between vestigial wings and yellow body color. Nor is there any association between vestigial wings and any other characteristic in the entire group that is linked with white eyes and yellow body. Clearly, vestigial wing is not a member of that group.

Another character that is independent of white eye color is black body color. In crosses which should test any such supposed relation, the distribution of black color of the body among the individuals is wholly unrelated to the white color of the eye. Black body occurs with equal relative frequency in individuals with red eyes and white eyes. Black body color is also independent of any other characters of the group linked with white eye color. But black body color *is* associated with vestigial wing. If a cross is made involving an individual with both vestigial wing and black body, then in generations produced by appropriate crosses among the descendants, black body and vestigial wing will occur together

much oftener than apart. That is, flies having both black body and vestigial wing will be relatively much more numerous than flies having black body and normal long wing; and much more numerous than flies with vestigial wing and normal gray body. Vestigial wing and black body color are clearly linked with one another.

With these two traits are also linked a number of others that concern the wings, the body, the eyes, etc. All characteristics linked with black body or with vestigial wing are, when tested in appropriate crosses, found to be linked with one another. They form a distinct second group, every member of which is linked to some extent with every other member. This group contains about as many characters as does the first group linked with white eyes. It must be made entirely clear that, while all the traits of this second group are linked with one another, none of them is in any way linked with any character of the first group to which white eye and yellow body belong.

There is still a third group of characters, and a fourth group quite small in numbers, which are made up, as are the first two, of characters that tend to hang together, once they start together. All members of the third group hang together more than mere chance would permit; and all members of the fourth group are in like manner associated with one another more frequently than can be attributed to accident. But no trait of the third group is in any way bound with any trait of the first, second or fourth groups. And no trait of the fourth group is linked to any extent with any member of any of the first three groups. *Three of these groups are rather large, that is, include numerous characters, one is quite small.*

Chromosomes and the Linkage Groups

You will have guessed long since that the reason assigned for the linkage of these various traits is that the hereditary factors responsible for them are located in the same chromosomes. All characteristics of the first group are produced by something in the same chromosome. All characteristics of the second group are likewise represented by something in *one* chromosome. But that chromosome is a different one from the chromosome that produces the characteristics of the first group. *Each of these groups owes its existence as a group to one chromosome, which is a different chromosome for each group.*

In this connection you will care to know something about the chromosomes of this fly. Fortunately they are well known. Each cell has eight of them (figure 3), but when, in the formation of germ cells, the reduction division divides these into two groups, there are only four in each germ cell. *Three of these chromosomes are large, and one quite small, and three of the linkage groups of characters are large, and one small.*

Assuming that the hereditary factors for one group are all in one chromosome, and that that is the cause of their linkage with one another,

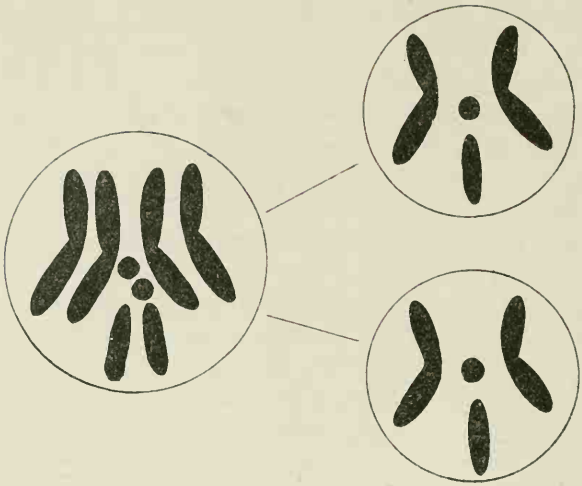

Figure 3. The chromosomes of the female fruitfly *Drosophila melanogaster*. In the body cells and immature germ cells there are eight chromosomes, two each of four kinds. In maturation, at the reduction division, the number is reduced to four, one of each of the four kinds. Three of the chromosomes are large, and one is small.

what becomes of the idea of individuality of the chromosomes? It must be modified, of course. As previously pointed out, the characteristics of each group are not absolutely bound together, they merely occur together more frequently than chance would permit in the case of *independent* characteristics. That is, once they are transmitted from parent to offspring in conjunction with one another, they separate from one another thereafter less frequently than would be expected. But if their factors are in the same chromosome, how can they separate at all?

Breaking the Linkage Groups

To make the proposed answer to this question clear it must be stated that when this separation does occur, there is a fairly even exchange. That is, when white eye is separated from yellow body with which it had been associated, some other eye color takes its place and is thereafter as closely linked with yellow body color as was the white eye color before. This is always the case. *Whenever a trait is removed from association with another trait, its place is taken by a trait related to the same part of the body.* Eye color is exchanged for eye color; one form of wing is replaced by another form of wing.

This exchange is made possible, presumably, because of the approximate duplication of the chromosomes in each cell. It has already been pointed out that the chromosomes of a cell may be such that they are exactly alike, two by two (figure 1). But even where the chromosomes are all

different from one another (figure 2), nevertheless they can be arranged in pairs of twins such that the members of one pair differ from one another in only one or a few features, but are alike in a host of others. One of them may, for example, include a representative of vestigial wing, the other of the normal long wing, but be alike in everything else. Or they may differ with respect to color of body and color of eye, and be alike in all other respects. The two chromosomes have to do with the same parts of the body, and no other chromosomes of the cell are concerned with those traits in the same way. The chromosomes are truly capable of arrangement in pairs of twins.

Mechanism of Crossing-over

This arrangement in pairs is not purely a figurative one, it is at certain times an actual bodily one. At a certain time in the formation of the germ cells, these chromosomes come together side by side. What they look like in this operation, is known in relatively few forms. In one of these the chromosomes are long slender threads, and the two twins twist about one another in loose spirals (figure 4). This is not an isolated case. It is

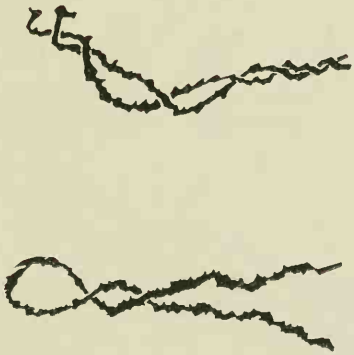

Figure 4. Some of the chromosomes of Batracoseps twisting about one another in spiral form prior to the reduction division. The chromosomes thus twisting together contain factors for the same hereditary characters. Whether they untwist in the reduction division, or separate in some other fashion, is not known from observation. (*Modified from Janssens.*)

not impossible that, in many or most animals, the twin chromosomes twist about one another at this stage of development. Later they separate from one another in some fashion at what we have called the reduction division. How this separation takes place is not known from direct observation, but several possibilities exist. The chromosomes may *unwrap completely* and be the same chromosomes as before they twisted. *Or they may adhere at points, and the two sides of the spiral in that region exchange places.* This is a very important conception, put forward by Professor Morgan and his students, but before it can be developed certain other considerations must be presented.

The hereditary factors contained in a chromosome are, Professor Morgan believes, *arranged in linear order*, like beads on a string (figure 5). Every cell in an animal's body has a chromosome in which these "beads" are the same as those of one chromosome in each of the other cells of the

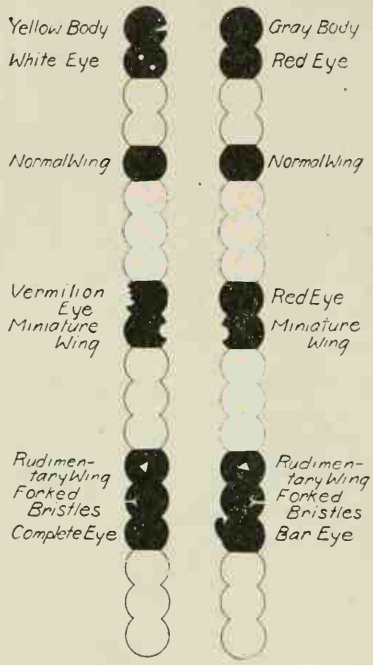

Figure 5. Diagram of a pair of chromosomes of the female fruitfly *Drosophila melanogaster*. The hereditary factors are arranged in a single row in each chromosome. Both chromosomes have hereditary factors for the same characters. The factors for one character are placed at the same level in both chromosomes, so that when the chromosomes meet in a pair the two homologous genes are side by side. Not all the known factors are represented. (*From Principles of Animal Biology, by Shull, La Rue and Ruthven. McGraw-Hill Book Co.*)

body. In the same cell with it is another chromosome in which the hereditary factors are precisely the same, or at least they concern the same parts of the body. The hereditary factors in these two chromosomes are held to be *arranged in the same order, and to lie at the same level.* So that, if the chromosomes are placed side by side, or twisted about one another, *the two hereditary factors for the same part of the body are side by side.*

If two such chromosomes twist about one another, as has been described, and then in separating are not unwrapped carefully, they may exchange hereditary factors. If in one of these chromosomes were a factor for white eye and one for sable body color (figure 6), some distance apart so that the breaking point occurred between them, the linkage that formerly existed between these two characteristics would be broken. Where they

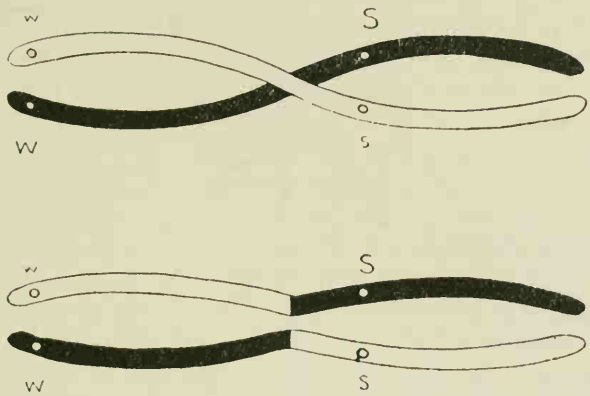

Figure 6. Crossing-over between two chromosomes containing factors for eye color and body color. One chromosome has factors for white eye (*w*) and sable body (*s*); the other has factors for red eye (*W*) and gray body (*S*). If these chromosomes adhere and break at some point between the pairs of factors, after the reduction division one chromosome contains factors for white eye (*w*) and gray body (*S*), the other has factors for red eye (*W*) and sable body (*s*).

had formerly necessarily passed to the same individual, they would now necessarily pass to different individuals. There is also much to prove that the chromosomes may break twice, or at three places instead of only one. If one of the two chromosomes that twist about one another (figure 7) has

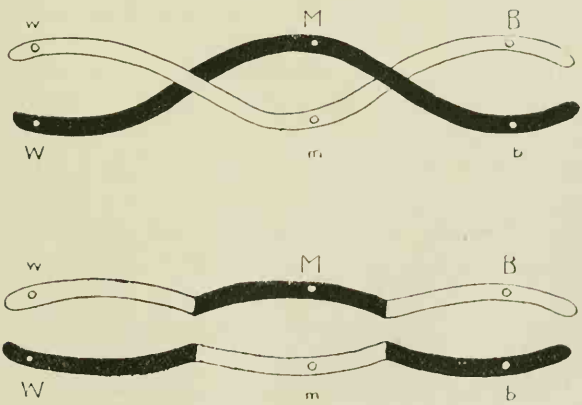

Figure 7. Double crossing-over in a pair of chromosomes. The chromosomes adhere and break at two points in their length, so that after the reduction division each chromosome is made up of three fragments, two from one of the original chromosomes, one from the other. In the original chromosomes the factors *w*, *m* and *B* were linked, as were also *W*, *M* and *b*. After the crossing-over, *w*, *M* and *B* form one linked group, *W*, *m* and *b* the other. See text for explanation of the symbols.

in it factors for white eye (*w*), miniature wing (*m*), and bar eye (*B*), the other the contrasted normal characteristics which are red eye (*W*),

long wing (*M*), and round eye (*b*), and these chromosomes break at two points as they separate from one another, two of the three linked characters may be still linked together, but the third separated from them. The separation of a hereditary factor from another with which it was linked is called crossing-over.

Whether a pair of twisted chromosomes shall break at one point or another is, with certain restrictions, held to be a matter of chance. If they break between the factors for black body and vestigial wing, those factors will be released from linkage with one another; that is, crossing-over between these two characteristics occurs. These chromosomes may break at any number of places *not* between the factors for black body and vestigial wing, and the two traits will remain linked as before.

Mapping Chromosomes

Inasmuch as breakage presumably occurs at different places in haphazard fashion, *crossing-over between two traits is likely to occur often if their factors are far apart on the chromosome.* Conversely, *if the factors are very near together they are seldom separated.* It is very easy, by making appropriate crosses, to pick out immediately those individuals in which certain characteristics, usually associated, have been separated, and hence in whose chromosomes breaking has occurred in a given region. By counting these individuals one may ascertain whether crossing-over between vermilion eye and club wing, for example, is frequent or rare; and can judge, therefore, whether the factors for these characteristics are far apart or near one another. By means of such experiments, it has been shown that white eye and yellow body seldom separate; they do so in only one out of a hundred chances, whereas they should cross over fifty times out of a hundred if they were independent of one another. Black body and vestigial wing, on the contrary, separate much more frequently, that is, about seventeen times out of a possible hundred. White eye and yellow body must therefore be very close together, black body and vestigial wing must be rather far apart. On the basis of such computations entire chromosome maps have been prepared. Such maps have been in existence for years, having been gradually developed, and altered as new evidence is procured. An abridged map of one of the chromosomes of the fruitfly is given in figure 8.

As new characters appear in this species, experiments are performed to determine in which chromosome their factors are, by determining with which other characters they are linked. And when that chromosome has been discovered, the place in it occupied by the new factor is next to be found. In locating the new factor, it may be necessary to alter the supposed place of certain other factors. That has happened time and again, for at first the location can be only tentative.

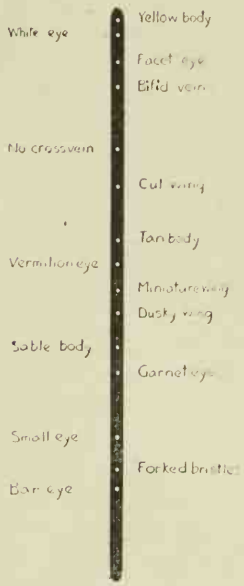

Figure 8. Abridged map of one of the chromosomes of the fruitfly *Drosophila melanogaster*, showing approximately the distances between the factors. The distances are determined by the number of times crossing-over occurs between the hereditary factors. Attention is especially called in the text to the distance between the factors for vermilion eye and sable body, and that between garnet eye and forked bristles.

Linear Order of Factors

While crossing-over can be easily demonstrated, it is admittedly pure hypothesis that it is accomplished by twisting of the chromosomes. It is also pure hypothesis that the factors are arranged in linear order, and that the amount of crossing-over between any two depends on the distance between them. However, any hypothesis is valuable in proportion to the number of things it explains, and a hypothesis that explains many things and is contradicted by nothing, has traveled a long way toward proof. Judged by these criteria, let us examine the situation of the hypothesis of the linear order of the hereditary factors and the twisting of the chromosomes.

First, assume that the amount of crossing-over between two characters, A and B, has been determined, and that from this amount the supposed distance between the factors for A and B, in terms of some arbitrary unit of measurement, has been computed. Suppose also that the distance between B and a third factor C has been similarly determined. If these determinations are actually computations of distances, then these two calculations fix the distance also between A and C, and it should be possible

to predict how great a proportion of crossing-over should occur between A and C, before the experiments to test it have been performed.

These predictions, when applied to short distances, have proven remarkably accurate. For example, as has been stated, in the first chromosome, breaking of the chromosome between the factors for yellow body and white eye occurs in about one per cent of possible cases. Crossing-over between white eye and bifid wing vein has been computed to occur in about five per cent of possible cases. If the map of this chromosome has been properly constructed (figure 8), crossing-over between yellow body and bifid wing vein should occur in about six per cent of cases. Within a small fraction of a per cent, this prediction is verified by experiments directly testing it. Similar verification has been obtained in numerous other groups of factors, and in no case where only small distances are involved have there been any serious discrepancies.

Errors in Long Distances

The fact that only in cases where small distances are computed is the correspondence between prediction and fact very close may at first seem to weaken the evidence in favor of the theory. On the contrary, *lack of close correspondence between prediction and discovery where long distances are concerned, is an important part of the confirmation of the theory.* I have already pointed out that the same two chromosomes, when they meet, may break at several points. Suppose that, in this case, the two chromosomes differed from one another in only two factors, white eye and bar eye (see figure 7, *w* and *B*), and that there were no identifying factor between them. If, in these premises, crossing-over should occur at two points between the factors for white eye and bar eye, the two factors would nevertheless be in the same chromosome after the chromosomes separated, and the experiment would not reveal the fact that crossing over had occurred at all. Crossing-over might occur four times, or six times, or any *even* number of times, and the two factors would still be left in the same chrommosome. When the two factors are separated by long distances, multiple crossing-over between them is likely to occur, *and only a part of it is detected.* Such factors would appear, by the usual computations, to be much nearer one another than they actually are. When a new factor is discovered, its location may be tentatively determined with reference to some other well known factor. If it turns out to be rather far from the factor first chosen as a zero point, the test may be repeated with another known factor which is probably near it. It has invariably happened that these later computations increase the distance as determined from the first long-distance test. That is simply because, in the second computation which concerns only a short distance, the investigator is discovering *all* the cases of crossing over, instead of only a fraction of them, and the dis-

tance is necessarily made to appear greater. So regularly has it happened that first computations of long distances have had to be increased as intermediate short distances are determined that the investigator is now able to guess with considerable accuracy how much a long distance diverges from the truth, and to fix with a good deal of precision the probable location of a new factor before the second computation is made. Lack of precision in the calculation of long distances is not, therefore, a weak point in the argument. Indeed, *there would be something the matter with the theory if long distances could be determined as accurately as short ones.*

Points of Cross-Over Never Near Together

The theory of linear arrangement of the factors, and of the twisting of the chromosomes, carries with it certain corollaries. It is scarcely possible that crossing over at one point of a chromosome is entirely independent of crossing over at other points in the same chromosome. Thus, if two chromosomes break at two points, a chunk is removed from the middle of each chromosome and transferred to the other. The size of this chunk is probably not entirely free of limitations. It extends from one point of crossing over to the other. How near or how far apart these breaks can be may depend on how tightly the chromosomes are twisted, or at how frequent intervals they adhere intimately to one another. No upper limit is set to the distance between cross-overs, but a lower limit is certainly to be expected. The size of a piece of chromosome that can be removed, by this method, to another chromosome presumably can not fall below a certain minimum. That means that *if breaking occurs at one point, it is not likely to occur at a nearby point at the same time.*

Fortunately, owing to the large number of factors now known in some of the chromosomes, this interference of crossing over at one point with crossing over at a nearby point can be tested. Thus, in one of the chromosomes (figure 8) the factors for vermilion eye and sable body color are at such a distance from one another that crossing over between them occurs once in ten times—in ten percent of cases. In a nearby region of this chromosome are two other factors, one for garnet eye, the other for forked bristles, so placed that crossing over occurs between them in twelve per cent of possible cases. If the breaking of the chromosome in one point has no bearing upon its breaking at another point, any chromosome that is severed between vermilion and sable should have twelve chances in a hundred of breaking also between garnet and forked. But experiments on a large scale show that, if crossing over occurs in the vermilion-sable region, it occurs also in the garnet-forked region in only a little over one case (1.2 to be more precise) out of a hundred. In like manner, if the various breaks of the chromosome are wholly independent of one another, out of a hundred chromosomes that have broken between

garnet and forked, ten should also be found to have broken between vermilion and sable. As a matter of fact, only one of the hundred breaks in the latter region. *Crossing over in one of these regions obviously interferes with crossing over in the other region.*

These two regions are near one another. The reason for the interference may be attributed, whether correctly or not, to the closeness or looseness of the spiral winding of the chromosomes about each other. When the two chromosomes adhere at one point, unless they are very tightly wound, they are not likely to adhere at another point except at some distance. If this explanation is the correct one, interference of crossing over should be most marked when the two regions are near one another, less marked when the regions are farther apart. That has indeed proven to be the result. Regions farther apart than these have been tested for simultaneous crossing over, and the degree of interference has decreased as the distance between the regions tested increased, up to a certain limit. This limit was reached when the two regions studied were far enough apart to account for about 46 per cent of crossing over between them. When regions farther apart than this were studied, then interference became greater again.

Variation in Frequency of Crossing-over

The idea that the frequency of crossing-over within a certain region depends upon the tightness with which the chromosomes twist has found expression in the explanation of another phenomenon. When this frequency of crossing over is determined by experiments, the results usually have a considerable degree of uniformity. The amount of crossing between vermilion and sable, for example, has never diverged very much, in an experiment involving large numbers of individuals, from ten per cent. However, certain strains of these flies have been found in which the ratio of crossing over differs very considerably from that found in other stocks. It is fairly uniform within the aberrant stock, but is different from other strains. This capacity for, let us say, a smaller amount of crossing over is, furthermore, transmitted to the offspring as a permanent family character. One interpretation put upon this phenomenon has been that some inherited feature, doubtless of a physiological nature, causes the chromosomes, when they meet in pairs, to wrap more loosely, about one another; or perhaps merely to adhere less frequently.

Further elaborations of this hypothesis of linear order of the hereditary factors, and of the twisting of the chromosomes, would be available if desired. Perhaps, however, the purpose which this lecture is designed to serve has been fulfilled. If it has shown that *the study of heredity has undergone a very considerable change in the past decade*, it has accomplished one of its aims. If instead of composing you to slumber it has convinced you

that the modern study of heredity is no longer a subject with which to lull oneself into an afternoon nap, it has attained another of its objects. I have not conducted you quite to the limits of present knowledge. Certainly we have not been anywhere near the confines of speculation. The discussion should have shown that in recent years the complexity of fact and theory in heredity has enormously increased. The progress made in this period is, I believe, *unquestionably proportionately greater than has been made in the same period in either physics or chemistry.* Biology, in at least this one division of it, has taken a long stride toward destroying the significance of that relative term by which the sciences of physics, chemistry, astronomy, etc., are so often fondly designated by their followers, namely, the "exact sciences." So complex now are the known phenomena of heredity, and yet in such close agreement are the multitude of facts of experiment and observation with the chain of hypotheses developed to explain them, that one who would contemplate the harmony of the universe may now reasonably strain his ear to catch, not the music of the spheres, but the concert of the chromosomes.

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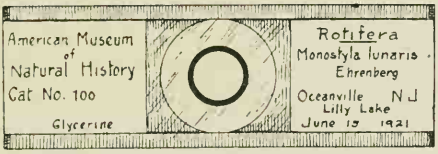
DEPARTMENT OF METHODS, REVIEWS, ABSTRACTS, AND BRIEFER ARTICLES

A NEW MICRO-SLIP

It is hardly necessary to add anything to the illustration of this convenient, strong and economical micro-slip, as it almost explains itself.

The body of the slip is made of sheet tin cut into parallelograms three inches long by one and one-quarter inches wide. A circle about thirteen-sixteenths in diameter is then punched out of the exact center and one-eighth inch on each long side is bent over forming two flanges into which the three finished units are slipped.

Object is mounted on a 25 mm square cover-glass of medium thickness in the regular manner. As the 25 mm squares are not very strong when handled by themselves, it is better to fasten them to a square piece of clear glass, before mounting, by squeezing down while wet, and then drying the upper side. This will give the necessary strength and support and they will be held firmly by capillary attraction.



When the mounting is completed, the 25 mm square is slipped into place at center of tin holder and a square piece of bristol board or card board slid into each end. The corners of the flanges are then squeezed tight with pincers and slide labeled.

A drop of transparent cement drawn under the flanges by capillary attraction, after the mount is completed, holds everything firmly in position and prevents any slight future movement of the units.

These slips can be easily made by any tin-smith.

The finished product is much stronger than the ordinary glass slip and the mount will seldom break on falling to the floor.

A ventral view of the mounted object can be had, just as easily as the dorsal view, by simply reversing the slide.

I am indebted to my friend Mr. H. K. Harring of Washington, D. C. for this slip originally, and have been using them for some years with every success.

The collection of Rotifera, about 500 species, in the American Museum of Natural History, New York City, are mostly mounted on these slips and have proven successful in all particulars.

FRANK J. MYERS.

Research Associate in Rotifera,
American Museum of Natural History

Since the above was written I learn the slip described is the invention of Dr. N. A. Cobb, Washington, D. C. It may be purchased from the Spencer Lens Co., Buffalo, N. Y.

KILLING, STAINING AND MOUNTING PARASITIC NEMATODES*

By
H. G. May

In some work on *Heterakis papillosa* of the domestic fowl I found that the ease with which fluids will penetrate the cuticula depends much on the method by which the worms have been killed.

Parasitologists are mostly following Looss in killing nematodes by means of hot solutions, usually of alcohol. This has the advantage of straightening the worms out as they die. The straightening seems to be due to the heat, as hot water will have the same effect. Cold concentrated killing solutions will usually cause the worms to die in very much contorted shapes.

When nematodes are killed by either of the above methods staining is rendered very difficult and mounting in balsam usually is impossible unless the cuticula is broken in one or more places. The final mounts are usually of a very inferior nature even tho all possible precautions are taken in changing from one fluid to another.

This resistance to penetration is not a normal function of the nematode cuticula as in the living worm fluids seem to penetrate the cuticula very readily. The resistance is due to a change in the character of the cuticula. This change is also indicated by the curling up or rolling up of free parts of cuticula such as wings or a pointed tail. These parts behave as tho the cuticula becomes semifluid during the killing process and then sets in an impervious condition.

This change can be prevented to a great extent by employing the method used by Dr. Cobb in killing free living nematodes. The essential point seems to be to start with a very weak solution. The nature of the chemical substance used is undoubtedly also of great importance. I have obtained very good results only with ethyl alcohol. Methyl alcohol and a solution of mercuric chloride both gave very inferior results; but this may have been due to the use of too strong solutions. Not only does this method prevent the change in the cuticula, but it also usually gives just as straight specimens as those killed in hot solutions.

In my work I still find it more convenient to use the string siphon differentiator as first described by Magath (Trans. Amer. Micr. Soc., 35: 245-256) and with the modifications described by me (Ill. Biol. Mon-

*Contribution No. 289 of the Agricultural Experiment Station of the Rhode Island State College.

ogr., Vol. 5, No. 2, p. 13) than to use Dr. Cobb's apparatus. An attempt to use his differentiator almost invariably results in the loss of valuable material because it cannot be found after the process has been completed. If the material is abundant so that the loss of a few specimens is of no consequence his method has the advantage of using less fluid and occupying less space. I find difficulty also in regulating the flow of liquid in his differentiator as that depends not only on the size of the capillary outlet but also on the weight of the column of liquid and the concentration of the alcohol, and the capillary tube may become clogged at any time.

In the string siphon method the specimens may be placed in a dish the bottom of which can be examined everywhere by means of a magnifying glass, binoculars or even a very low power of a compound microscope. The specimens need not be disturbed during the entire process of dehydration and clearing. By the method I am now using a single nematode of microscopic dimensions may be placed in a deep embryological watch glass while alive, and killed, stained, dehydrated, cleared and infiltrated with balsam without being disturbed. The only transfer necessary is that from the watch glass to the final mount on the slide.

By this method the specimens are placed in a watch glass or small stender dish in eight to nine tenths per cent salt solution. By means of a string siphon an amount of 5-7 per cent ethyl alcohol equal to four or five times the volume of salt solution is passed over the specimens. This is followed by an equal amount of 10 or 15 per cent alcohol and so on. Each time the concentration of the alcohol is increased by 5 to 10 per cent. The size of the string should be so regulated that it requires from 6 to 12 hours for one change of fluid to take place.

The staining may be done anywhere in the course of the dehydration. I usually stain with Delafield's or Boehmer's hematoxylin in 70 per cent alcohol with the addition of a small amount of acetic acid to aid penetration and to avoid the necessity of destaining. This is followed by a weak solution of sodium or potassium acetate in 75 or 80 per cent alcohol. The dehydration is then continued.

Absolute alcohol is followed by 1-3 xylene in alcohol, 2-3 xylene in alcohol and pure xylene. If specimens are to be sectioned they are now imbedded in paraffin by the method described in my paper quoted above. If they are to be studied as cleared specimens or mounted in balsam they are next passed from xylene to synthetic oil of wintergreen just as they were passed from alcohol to xylene. This gives the specimens a maximum amount of clearing and makes excellent preparations for study at that time. Mounting in balsam renders the specimens more opaque and makes them appear more like they did in xylene.

To mount in balsam it is usually best to infiltrate the specimens first by removing all but a thin layer of the oil and then adding a small

lump of dry Canada balsam every day or so until the solution has acquired about the consistency of the balsam to be used for the mount. For permanent mounts it is best to use balsam dissolved in xylene even tho this does not retain the transparency of the object because balsam in oil of wintergreen requires months to harden. Gum dammar may be used for mounting but not for the process of infiltration as it does not dissolve completely in the methyl salicylate.

A NEW LOCALITY FOR *SPONGILLA WAGNERI* POTTS

The description of this species in 1889 was based on material collected in a creek on the southwestern coast of Florida. The sponges were found encrusting barnacles and tubes of Serpulae. The presence of these marine forms in a fresh-water creek was assumed to be due to occasional backing up of salt water from the Gulf under the influence of strong southwest winds. I have found no reference to the occurrence of the species elsewhere in North America, but it has been recorded as occurring in Brazil.

Fresh-water sponge material recently received from the Southern Biological Supply Co. and collected in Lake Pontchartrain, near New Orleans, includes specimens of *S. wagneri*, and again the colonies are associated with barnacles. P. Viosca Jr. has informed me that under certain conditions salt water gains entrance, and varying degrees of salinity occur at times in different parts of the lake. It seems probable that representatives of the species may be found in suitable localities in other states along the Gulf Coast.

FRANK SMITH.

University of Illinois.

SOME INTERESTING STUDIES ON SPIDER ANATOMY

By
E. W. Roberts

On studying the spider Anglena I was struck by the lack of conformity of the material with descriptions of the class given in prominent text books. For instance, in Parker and Haswell's Zoology, vol. 1, page 615, the pedipalpi of spiders is described thus: "The pedipalpi (Fig. 506, B) are elongated, and end in simple extremities; in the male (Fig. 507) the *terminal* joint is modified to serve for the reception and transference of the sperms." In the pedipalpi of the male Anglena, we find the third from the end, not the terminal joint, serving as a very highly modified intromittent organ. The pedipalpi (Fig. 1) are composed of at least 9 separate segments as I have numbered them from the extremities inward. Segment 1 is modified into a terminal clasper, or claw. Segment 2 is a much enlarged gland and olfactory joint; there are two large areas of perforated openings, one of which gives off sexual odors, while the smaller perforated area consists of olfactory cells. This joint during sexual contact is placed directly over the stigmati or air chambers which open into the book-lungs. Thus in this case the book lungs function in an entirely new light as olfactory organs. Segment 3 is modified into a very complicated intromittent organ, which is shown greatly enlarged in photo No. 2.

In preparation for mating, the male spins a mat of web which is held under the sexual vents and the sperms emitted thereon. This web with its contents is then brought forward and the end of the joint marked A is inserted in the fluid mass which is sucked through slit apertures into the large coiled storage glands on which I have indicated the direction of the flow by arrows. The large gland makes about one complete turn and ends in a minute tube which terminates outward in the true intromittent organ marked B. During copulation the male, watching his chance, rushes behind the female and grabs her around the neck of the abdomen and inserts the terminal B in the small vaginal vents which are shown as a pair in photo No. 3 and marked A-A.

The stigmati c-c are situated external to the vaginas and are marked B-B. In this position the large gland organs of joint 2 come into position over the stigmati of the further side, while joint 1 clasps its claw further up the side.

Segments 4-8 are well represented in the illustration. Segment 9 is enlarged (Fig. 4) forming two lips which contain the most highly developed nervous system of any organ in the body.

The nerve cord spreads out fan-wise, divides into thousands of terminals which end in the minute cuticular hairs or spines. These hairs dip into the blood of the victims and are probably gustatory in function.

In Parker and Haswell's Zoology, vol. 1, page 612, order 6, Araneida. "Arachnida in which the body is composed of an *undivided* cephalothorax and an unsegmented abdomen." The claims of most textbooks on the "undivided head and thorax" of this class, may suit the men who proposed the term of cephalathorax, but to students of anatomy it is more or less unfounded as I will try to show. It is doubtful if the head structures and the thorax structures have ever coalesced or fused as some casual outer views would indicate. The neck of spiders is as large as the thorax and is doubtless made so by the fact that in bulk with its appendages it is as large as the true thorax. I have no doubt that the full sized neck of spiders more nearly approaches the ancestral types of Arthropoda than that of Diptera which are reduced in some cases to a mere stalk. But this would not keep a spider from having as true and anatomically complete head as a fly or bee. Fig. 5 is a long vertical section of the head and thorax of a female Anglena spider. The line marked E is the true division of the head and thorax structures, there is no sign of structural coalescence. In the thorax, figures 1-4 indicate the four legs and the corresponding muscles which move them. A glance will show the head and thorax structures nearly balanced in bulk, but entirely distinct regionally. The pedipalpi (A) form the lower lip, the lower neck fold being between this and the fourth thoracic leg. B is the relatively enormous chelicerae, the eye and the poison gland of the chelicerae are represented at B, C, and D. Fig. 6 shows the head of a male Anglena spider and several additional features. On the side of the head prominent folds of neck skin project forward above the eye C and is designated E. This may be the same neck fold which bending front or back is called a carapace. A is the pedipalp base and B the chelicerae. The brain ganglia of the head (D) lie forward of and in close contact with the thoracic ganglia.

In spiders the nerve ganglia of the head and thorax form one long brain body, but there is no coalescence or fusing of the ganglia. The nerve trunks of all the head appendages still run to their proper brain lobes and are easily followed out in sectioned material. The only thing that has happened to the ganglia is the shortening of the trunks between ganglia and their approachment to each other.

In Parker and Haswell's Zoology, page 612, order 7, Acarida we find the following "Arachnida in which the body exhibits no division into regions." And on page 616 "In the Acarida or Mites and Ticks (Figs. 508 and 509 the distinction into regions is no longer recognizable." In my specimens of Trombidium fuliginosum there is a small but distinct fold on the ventral side between the abdomen and thorax, while the

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108a

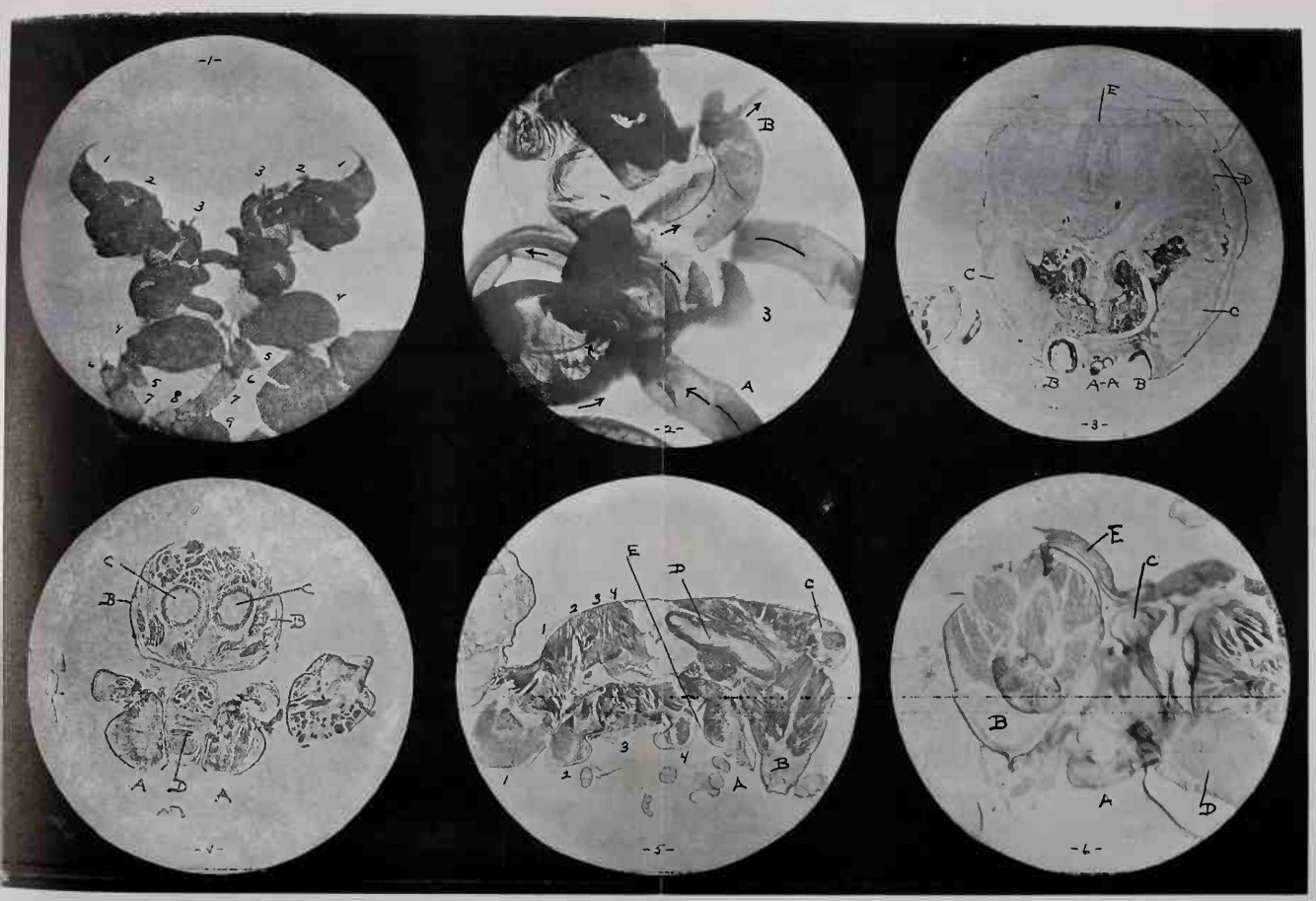

PLATE XII

stigma lie in their proper place just to the rear of the fold, the same position as they take in spiders. In the fig. 508 of the itch mite Sarcoptes scabiei which they give the abdominal fold and the stigma just to the rear are plainly in view. These points are of minor importance, but, if they are correct anatomy why would they not be correct for teaching purposes.

Explanation of Plate

Fig. 1, Anglena—male spider—organs pedipalpi; *1*, terminal claw; *2*, scent gland. *3*, intromittent organ. *4*, large oval clasping segment; *5*, *6*, *7*, *8*, small segments; *9*, highly developed segments which form lips for the mouth.

Fig. 2. Enlarged view of intromittent segment. *A* is the intake of the storage tube, the arrows indicating the direction of the flow. *B* is the outlet of the storage tube which is furnished with a shoulder to prevent too deep insertion.

Fig. 3. Section of anterior abdomen of female spider, cut through stigmas and vaginas. *A-A*, vaginal openings, *B-B*, stigmas; *C-C*, booklungs; *D*, liver; *E*, heart.

Fig. 4. Section through head of male spider. *A-A*, lips; *B-B*, chelicerae; *C-C*, poison glands; *D*, mouth.

Fig. 5. Long vertical section of head and thorax of female spider. *A*, lower lip. *B*, chelicerae; *C*, eye; *D*, poison gland; *E*, neck; 1, 2, 3, 4, legs and the muscles which move them.

Fig. 6. Long vertical section of head and thorax of male spider. *A*, lip or pedipalpi; *B*, chelicerae; *C*, eye; *D*, brain; *E*, neck fold or carapace.

ANNUAL REPORT OF THE TREASURER OF THE AMERICAN MICROSCOPICAL SOCIETY

December 17, 1920 to December 24, 1921

Receipts

Balance on hand December 17, 1920	$444.19
Dues received for Volume 39 or before	75.00
Dues received for Volume 40	200.00
Dues received for Volume 41	294.00
Initiation Fees	63.00
Subscriptions for Volume 39 or before	15.00
Subscriptions for Volume 40	220.60
Subscriptions for Volume 41	26.44
Sales of Transactions, duplicates, back numbers	121.01
Advertising, Volume 39	206.50
Advertising, Volume 40	10.00
Mr. Z. P. Metcalf, plates and reprints	41.85
Mr. F. B. Taylor, reprints	4.61
Total	$1722.20

Expenditures

Printing Transactions, Volume 39, No. 4	$108.07
Printing Transactions, Volume 40, No. 1	255.81
Printing Transactions, Volume 40, No. 2	230.32
Printing Transactions, Volume 40, No. 3	284.23
Printing Authors' Reprints	19.35
Postage and express for Secretary	23.13
Postage and express for Treasurer	7.50
Office expenses of Secretary	50.40
Office expenses of Treasurer	21.95
Office expenses of Custodian	4.75
Secretary, trip to Chicago Meeting	38.98
Spencer-Tolles Fund	85.00
Balance on hand	592.71
Total	1722.20

W. F. Henderson, *Treasurer.*

December 24, 1921.
Examined and found correct.

Raymond J. Pool
Robt. H. Wolcott
Auditing Committee.

March 27, 1922.

CUSTODIAN'S REPORT FOR THE YEAR 1921

Spencer-Tolles Fund

Balance reported for the year	$6617.66
Contribution	1.76
Interest and dividends	450.14
Sale of Transactions	85.00
Profit on change of investment	1950.00
Total	9104.59
Increase during the year	$2486.90

Totals

Receipts

All contributions	802.03		
All sales	1193.38		
All life-memberships	300.00		
All interest and dividends	5149.15		
Profit on change of investment	1950.00		
	9394.56		

Disbursements

All Grants	250.00		
All Dues for life-members	40.00	290.00	9104.56

Investments

Stock in Keystone State Building and Loan Ass'n of Pittsburgh, Pa	1954.56	
Bonds of Rio Grand Junction R'y	5000.00	
43 shares of stock Pa. R. R. Co	2150.00	9104.56

Life members: (Robert Brown, dec'd) J. Stanford Brown, Seth Bunker Capp, Harry B. Duncanson, A. H. Elliott, John Hately.

Contributions of $50 and over: John Aspinwall, Iron City Microscopical Society. Magnus Pflaum, Troy Scientific Society.

Magnus Pflaum, *Custodian.*

Philadelphia, Pa., Dec. 31, 1921.

Item "Interest and dividends" (1921) shows net receipts and accruals after payment of some minor expenses. Items "Profit on change of investments," and the inventory of "Investments," take the securities at their par value. Items in the B. & L. Assn. (pass-book) account of Jan. 1st, 1921 and Jan. 3rd, 1922 are taken in this account as of Dec. 31st, preceding, respectively.

Having examined the above account, in connection with cash account covering the same period, and having compared the receipts and expenditures shown therein, with the vouchers and with report for previous year, we find it correct.

F. E. Ives
Edward Pennock
Auditing Committee

112,

112 a

TRANSACTIONS

OF THE

American Microscopical Society

ORGANIZED 1878 INCORPORATED 1891

PUBLISHED QUARTERLY

BY THE SOCIETY

EDITED BY THE SECRETARY

PAUL S. WELCH

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VOLUME XLI

NUMBER THREE

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TRANSACTIONS
OF
American Microscopical Society
(Published in Quarterly Instalments)
Vol. XLI JULY, 1922 No. 3

NOTES ON THE EXCRETORY SYSTEM IN *ASPIDOGASTER CONCHICOLA**

By
Ernest Carroll Faust
Peking, China

Aspidogaster conchicola is a cosmopolitan parasite. It is commonly found in Anodon in Central Europe and is the most common parasite of the Unionidae in North America. It has at times been described from gasteropods and infrequently from fishes. Examinations from several distinct centers in China reveals the presence of the fluke in the following host species:

HOST	Specimens Examined	Per Cent Infected	Locality
Unio sp.	18	44.4	Peking
Vivipara lapillorum	2232	3.2	Peking
	440	6.6	Wuchang
	280	11.4	Changsha
Vivipara catayensis	112	1.7	Changsha
Leuciscus aethiops	17	100.0	Wuchang
Amyda sinensis	8	50.0	Wuchang
	20	5.0	Changsha

In the case of the lamellibranch the worms were found parasitic on the renal organ; in the gasteropods they were dissected out of the lymph sinuses of the liver or taken from the spermary. In both the fish and the turtle the worms occurred in the intestine.

The finding of *Asipidogaster conchicola* in several regions in China adds new records of distribution for the species, while the presence of the parasite in the intestine of *Amyda sinensis* allows one to record a new host.

*Contribution from the Parasitology Laboratory, Department of Pathology, Peking Union Medical College.

The external features of the worm and the digestive and reproductive organs have been adequately studied, particularly by Stafford (1896). From the material at my disposal I was able to develop certain facts regarding the excretory system which have previously been but poorly understood.

The Excretory System in the Adult Worm

This was studied by Stafford (1896: 506-516), who traced correctly the main portions of the system, and followed "a few branches out to the end organs." He states that "with this as a skeleton one can fill in mentally the rest of the branches with their funnel organs and get a fair conception of the semi-excretory system" (p. 511). From this study Stafford was able to hypothecate the "tri-radiate order of branching" and a "tolerably clear symmetry of arrangement." Although he was able to find a more exact symmetry than Huxley (1856) and Voeltzkow (1888), he still found an asymmetrical relation in the anterior branches on right and left sides. He states further that "if all the branchings are regular and the process is repeated six times, we can, by a simple calculation, estimate the number of funnel organs in a single *Aspidogaster*. Thus: $4\times3\times3\times3\times3\times3$ will give the number for one side and doubling this we get a total of 1944."

The apparent asymmetry which Stafford found in the anterior branches is not substantiated by my studies. Still more improbable is Stafford's calculation of the number of ultimate capillaries based on the study of "a few branches followed out to the end organs." Moreover, the irregularities which he notes in other parts of the system can only be interpreted as inadequate analysis. This is particularly true in the light of my studies which indicate *complete symmetry and regularity in the system of the larva as well as in that of the adult.*

While Stafford predicates four main branches, each of which proceeds to trifurcate successively five times, I have found only three such main stems and each of these in turn branches trichotomously only four times. Each of these branches has been traced to its distal termini, with the result that complete regularity is found to obtain (Fig. 1). In other words, the system for each side of the body actually consists of 3 stems, each branching four times, with 81 terminal units, giving a total of 243 capillaries and flame-cells for each side of the body. This, as against Stafford's mental calculation of 972 units which is theoretical and obviously based on inadequate analysis. The system may be expressed as $(3\times3\times3\times3)+(3\times3\times3\times3\times)+(3\times3\times3\times3)$ or $(3)^4+(3)^4+(3)^4$, and reduces to the formula $\alpha^n+\beta^n+\gamma^n$.

These data are supported further by the elemental excretory system which is found in the larva (figs. 5, 6). Here three primitive flame-cells and capillaries are seen which are the basal units of the system, $(\alpha+\beta+\gamma)$. These by successive trichotomies give rise to the adult system.

It seems desirable, in passing, to note that the larva (fig. 7) has paired cluster of cephalic glands (cg), emptying through a cord of cephalic ducts (cgd) just anterior to the oral sucker. This is analagous to the salivary glands described for the redia of *Cercaria equitator* (Ssinitzin 1911: 52, fig. 50) and *C. flabelliformis* (Faust 1918: 34, fig. 43) and might lend support to the view that the aspidobothrid is a redia with germinal epithelium highly differentiated.

Summary

1. Record is made of the presence in several centers in China of the cosmopolitan worm, *Aspidogaster conchicola*. In addition to the usual hosts, *Amyda sinensis* has been found to harbor it.

2. The excretory system of the worm is regular and bilaterally symmetrical. It has three main stems on each side of the body, each stem having a 4-fold trichotomy.

3. This is expressed as $(3\times3\times3\times3)+(3\times3\times3\times3)+(3\times3\times3\times3)$ or $(3)^4+(3)^4+(3)^4$ and may be reduced to the formula $\alpha^n+\beta^n+\gamma^n$.

4. The fundamental $\alpha+\beta+\gamma$ pattern obtains in the larva where each element is represented by a single flame-cell.

5. The presence of cephalic glands in the larva may support the view that the worm is homologous to a highly differentiated redia.

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Key to Figures

α,	anterior fundamental of excretory system.
β,	median fundament of excretory system.
γ,	posterior fundament of excretory system.
b, b_I, b_{II},	bladder.
cg,	cephalid gland.
cgd,	cephalic gland duct.
ct,	primary collecting tube.
ep, ep_I, ep_{II},	excretory pore.
rt,	reflexed (secondary) collecting tubule.

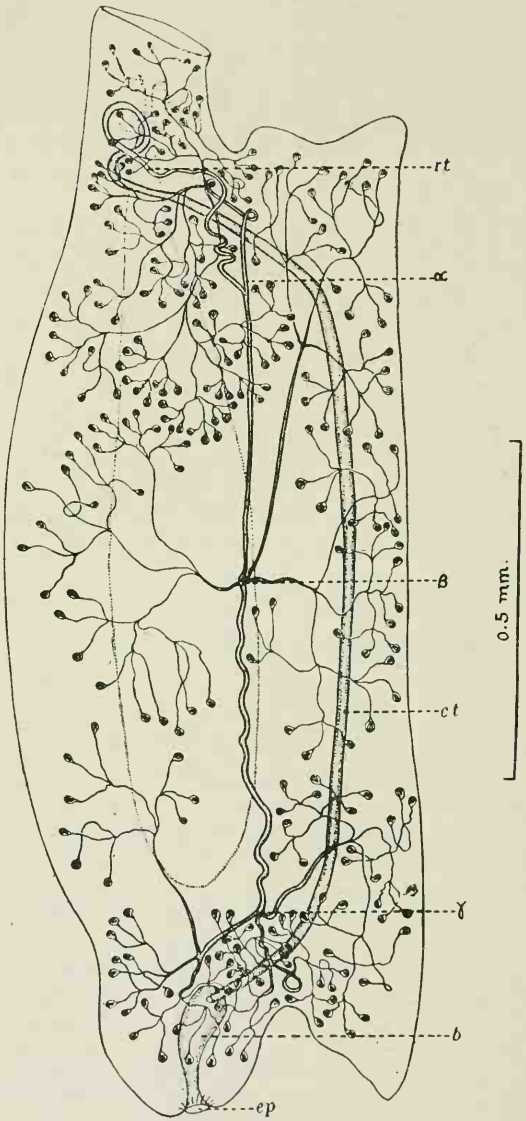

Fig. 1

PLATE XIII

Description of Figures

Fig. 1. Lateral view of adult *Aspidogaster conchicola*, showing complete excretory system for right side of body, X 75.

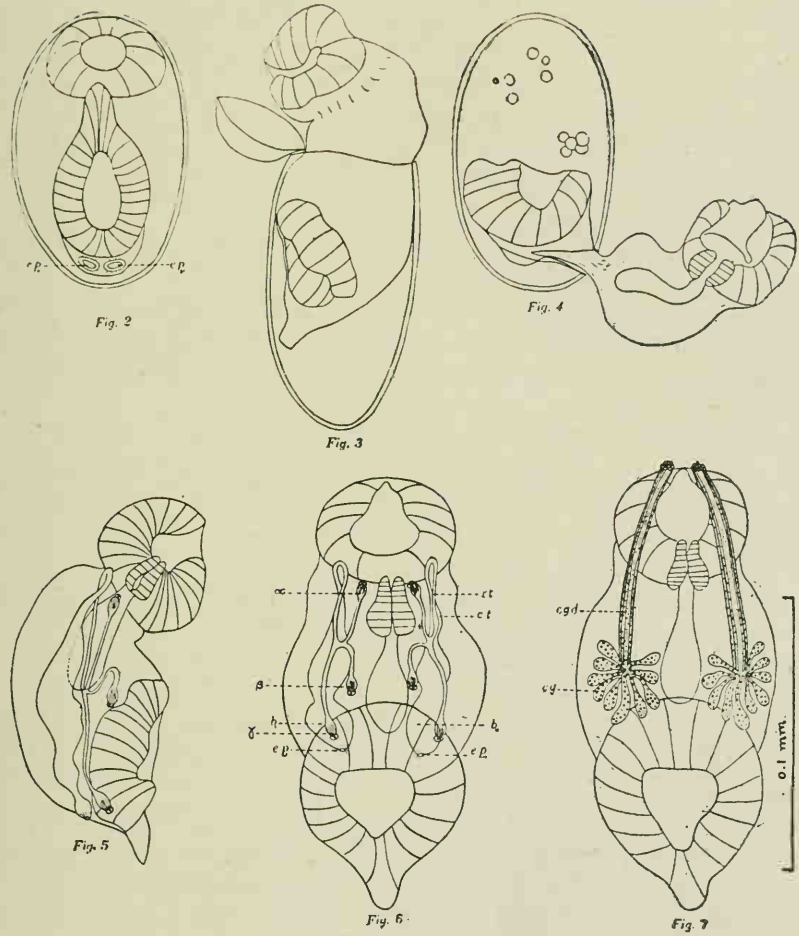

PLATE XIV

Figs. 2 to 4. Stages in the escape of the larva from the egg shell, X *360*.

Figs. 5, 6. Lateral and ventral views of the free larva, showing fundaments of the excretory system. The two sides are seen to develop separately, even to the excretory pores. X *360*.

Fig. 7. View of larva showing cephalic glands and ducts, X *360*.

A NEW CESTODE FROM LIPARIS LIPARIS*

By
Edwin Linton
University of Missouri

The name *Spathebothrium simplex* gen. et sp. nov. is proposed for a cestode collected at Woods Hole, Mass., by the late Vinal N. Edwards from the sea snail, *Liparis liparis*.

Mr. Edwards's record is as follows:

March 25, 1904, 2 fish examined, stomachs filled with small sand fleas, 2 tape worms in intestine of one.

April 14, 1904, 15 fish examined, stomachs filled with sand fleas; full of spawn, nearly ripe; 7 tape worms from 4 fish.

January 14, 1905, 2 fish examined, one tape worm in each.

The cestodes in these three lots belong to the same species. Their lengths, in alcohol, were 12, 14, 15, 16, 18, 18, 20, 20, 21, 26 and 33 millimeters respectively. The maximum breadth was about 2.25 millimeters; ova 0.036 by 0.021 mm. in the two principal diameters.

The strobile is flattened, nearly linear, bluntly and smoothly rounded at the two extremities. About the only difference noted between the anterior and posterior ends, as seen in whole mounts, is that genitalia are wanting for a short distance at the anterior end while the vitellaria continue to the extreme tip of the posterior end. In a specimen 11 mm in length the first cirrus is 0. 44 mm. from the anterior end. The scolex therefore is represented by the short portion which precedes the genitalia and is probably transparent in life. In this specimen the vitellaria began a little posterior to the level of the cirrus.

The strobile is not divided into distinct proglottides, the only indication of strobilation being the successive sets of genital apertures, and, in cleared and mounted specimens, the ovaries which are conspicuous, lobed, and lie between the genital apertures at what would be the posterior end of a proglottis, if proglottides were present.

The reproductive apertures are situated along the median line and are not restricted to one of the flat surfaces of the strobile. For example, in a specimen which had 20 sets of reproductive organs twelve of these opened on one of the flat surfaces of the strobile and eight on the other. In another specimen sixteen were counted on one side and nine on the other. In a specimen 16 mm. in length the distance between adjacent sets of reproductive pores was about 0.67 mm., the first set lying about the same distance from the anterior end. The apertures of the cirrus, vagina and uterus are

*Contribution from the U. S. Biological Station, Woods Hole, Mass., and the Zoological Laboratory of the University of Missouri.

near together, that of the cirrus being a little anterior to those of the uterus and vagina, which are very near together and about at the same level.

The vitellaria continue without interruption from near the anterior end to the posterior end, so that the strobile superficially resembles an elongated trematode. The testes lie for the most part in front of the ovary and are medially placed with respect to the vitellaria. In transverse sections through regions where the uterus is filled with ova the testes are lateral and near the vitellaria (Fig. 6). The cirrus pouch is short but with relatively thick muscular walls. The vagina has a strong muscular sphincter near its external opening (Figures 2, 4, 5). In ripe strobiles each set of reproductive apertures is preceded by a mass of ova.

The musculature, so far as it is shown in sections, is poorly developed. A few longitudinal fibers were noted in the subcuticula, but no trace of a layer of longitudinal fibers between the subcuticula and central parenchyma was seen, nor was there any indication of a circular layer. The cuticle (Fig. 3) consists of two layers, an outer made up of short rod-like structures, and an inner structureless layer. The outer layer constitutes about two-thirds of the thickness of the cuticle but it may be more or less abraded. The subcuticula in my sections appears as a loose mesh of fine fibers with scattering cells. The thickness of the cuticle in the section from which figure 3 was sketched was 0:01 mm., of the subcuticula 0:07, and of the smaller diameter of the section, representing the thickness of the strobile, 0.5.

Sections of the anterior end of a strobile show numerous anastomosing vessels of the excretory system. These vessels were difficult to interpret in transverse sections in regions of the strobile where the reproductive organs had appeared. Nowhere were they satisfactorily seen to be definitely established as dorsal and ventral lateral vessel. In cases where two principal lateral vessels could be distinguished they lay in about the same horizontal plane with reference to the axis of the strobile. From a study of a series of sagittal sections the lateral vessels were interpreted to be two, with thin walls, somewhat tortuous, and giving off transverse branches.

This cestode is peculiar in the absence of bothria, and in certain characteristics of the genital pores. The three genital apertures, cirrus, uterus, and vagina, are, as a rule, near together on the median line, and irregularly alternate with respect to the so-called dorsal and ventral surfaces of the strobile. This feature stands in the way of referring it to the *Pseudophyllidae*, which group is characterized by having the opening of the uterus always on one of the faces, although the openings of the cirrus and vagina may stand on opposite faces, or on a lateral margin.

It is thus seen that the species with which we are dealing is unique in that it is not possible to speak of a dorsal and ventral surface of the strobile. For it will be observed that, not only are the reproductive apertures irregularly alternate on the flat surfaces of the strobile, but the reproductive organs themselves are also irregularly alternate with respect to those surfaces (Fig. 2).

While examining a large number of transverse sections a single exceptional disposition of the reproductive apertures was noted. Figures 7 and 8 are sketches of this exceptional condition. Here the aperture of the cirrus is seen to be on one of the flat surfaces of the strobile while the openings of the vagina and uterus are on the opposite side. Since the apertures of the uterus and vagina do not lie in the same horizontal plane it was necessary to make two sketches. In the series of sections in which this anomalous condition was noted two sections intervened between the sections shown in figures 7 and 8.

An interesting feature with respect to the relative position of ovary and vaginal aperture is shown in figure 2. In the upper part of the figure the ovary is seen to be on the opposite side of the strobile from the vaginal aperture, in the lower part of the figure it is on the same side. In the former case the vagina crosses from one side of the strobile to the other, in the latter it turns abruptly posteriad near the aperture.

Synopsis of genus *Spathebothrium*

No distinct scolex; strobile taenaeiform, bluntly rounded at the extremities, proglottides not distinct, reproductive apertures on median line and irregularly alternate.

Explanation of Plate

c. cirrus
cp. cirrus pouch
cu. cuticle
m. sphincter muscle of vagina
o. ovary
sc. subcuticula
sg. shell gland
sr. seminal receptacle
t. testes
u. uterus
v. vagina
vd. vas deferens
vg. vitelline glands.

Fig. 1. Sketch of specimen mounted in balsam, somewhat diagrammatic. In this specimen there were 12 sets of reproductive apertures on one side and 8 on the other. Length 16 mm.

Fig. 2. Sagittal section near median line showing reproductive apertures on opposite sides of the strobile. The succeeding section to this in the series shows the uterus in about the same relative position as that of the vagina in the lower left of the sketch. Thickness of strobile at this point 0.30 mm.

Fig. 3. Cuticle and subcuticula highly magnified. Thickness of cuticle 0.01 mm.

Fig. 4. Reproductive apertures as seen in horizontal section. Sketch made from section showing first appearance of the uterus. The vagina had appeared first in the preceding section, and the cirrus in the sixth preceding section. Diameter of cirrus bulb 0.24 mm.

Fig. 5. External apertures of vagina and uterus, transverse section. Long diameter of ovum 0.035 mm.

Fig. 6. Transverse section showing uterus with ova, etc. Breadth of strobile at this point. 1.5 mm.

Fig. 7. Transverse section showing exceptional arrangement of genital pores, the cirrus opening on one side and the vagina on the other. Longer diameter of section 0.98 mm.

Fig. 8. From same series of sections as Fig. 7, two sections intervening between 7 and 8. The cirrus bulb still shows and the vagina is replaced by the uterus.

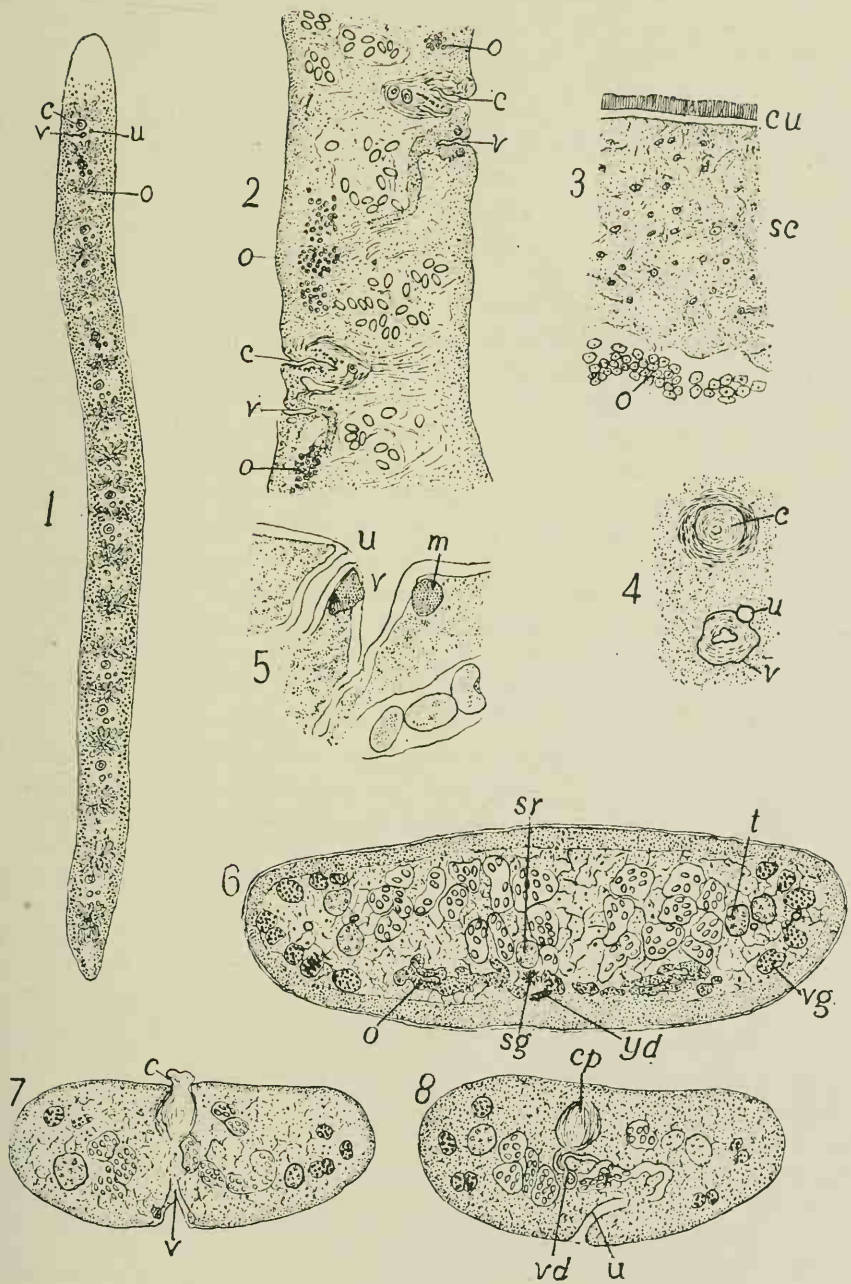

PLATE XV

A LIST OF THE NEW GREGARINES DESCRIBED FROM 1911 TO 1920*

By

Minnie Watson Kamm

Although the gregarines are among the oldest known of the Protozoa (Redi, 1684), they still remained a practically unknown group for a hundred and fifty years, researches on more recently described groups far outnumbering those on these parasites. This may have been due to the fact that gregarines are of little or no economic importance. The hosts as a rule are not animals of such import that the elimination of their parasites is desirable and, moreover, the parasites themselves are generally harmless, living commensal rather than actual parasitic lives within their hosts.

Because this is practically a new field, much of the work on the group has been up to the present chiefly systematic; it is often easier to find an entirely new species than to obtain a species already known. Considerable has been done on Life Histories, Effect of the Parasite Upon the Host, and Chromosome Behavior in the Complete Life-Cycle during the last decade and much more is to be expected in these fields.

Labbé described the gregarines known up to the year 1899[1] and reclassified many of the wrongly designated and aberrent species. His paper was probably the incentive for much of the subsequent work on the group. The new forms described from the time of Labbé's Summary up to the year 1911 were listed by Sokolow[2] and those described in this decade compared favorably with the complete summary of Labbé.

Because the number of new species has been rapidly increasing subsequent to Sokolow's List, the writer has prepared a list of the species described in the literature from 1911 to the beginning of the year 1920. During this decade were named many new genera and the genus Gregarina received many new species.

Perhaps the most important researches of the decade were those in the Suborder Schizogregarinae which includes many aberrent and apparently unrelated species and consequently the classification is considerably confused.

The classification which follows is that of Minchin, at present the best known.

* Contribution from the Zoological Laboratory of the University of Illinois, No. 204.

[1] Das Tierreich, Pt. 5, Sporozoa.

[2] Zool. Anz. 38:277-95; 304-14.

Class Sporozoa Leuckart

Subclass 1. Telosporidia Schaudinn 1900. Sporulation at end of vegetative period.

Order 1. Gregarinoidea Minchin 1912. Trophozoite parasitic in epithelial cells. Sporont free in a cavity. Spore forms a single zygote.

Suborder 1. Eugregarinae Léger 1900. Reproduction by sporogony only.

Tribe 1. Cephalina Delage and Hérouard 1896. With epimerite in trophozoite stage. Septate in all but one family. Generally parasitic in digestive tract of insects.

Tribe 2. Acephalina Delage and Hérouard 1896. Without epimerite, non-septate. Generally coelomic.

Suborder 2. Schizogregarinae Léger 1900. Reproduction by both sporogony and schizogony.

Tribe 1. Monospora Léger and Duboscq 1908. Single spore in sporogonic cycle.

Tribe 2. Polyspora Léger and Duboscq 1908. Many spores in sporogonic cycle.

An Annotated List of Species in the Tribe Cephalina of the Suborder Eugregarinae[3]

Family LECUNIDAE Kamm (1922).

Epimerite simple, symmetrical, gregarines non-septate, spores ovoidal, thickened at one pole. Digestive tract of marine annelids.

Genus *Lecudina* Mingazzini 1891

Characters of the family

Lecudina sp.

Faria, Cunha, and Fonseca (1918) Mem. Inst. Osw. Cruz, 10:17-19. Body spindle-shaped, nucleus spherical.

Host: *Polydora socialis* (Polych.) Taken at Rio de Janeiro, Brazil.

Family POLYRHABDINIDAE Kamm 1922.

Septate gregarines inhabiting the digestive tract of marine annelids. Epimerites varied.

Genus *Polyrhabdina* Mingazzini 1891

(See Caullery and Mesnil 1914 C. R. Soc. Biol., 77:516-20.) Dicystid, sporonts flattened, ovoidal, epimerite a corona of hooks. Intestine of polychaetes of the family *Spionidae*.

Polyrhabdina spionis (Kölliker) (New name for *Gregarina spionis* Köll.)

[3] All parasites described are intestinal forms unless otherwise stated.

Type species. Caullery and Mesnil (1914) C. R. Soc. Biol., 77. 516-20.
Host: *Scololepsis fuliginosa.* (Polych.)

Polyrhabdina polydorae (Léger) (New name for *Doliocystis p.* Léger.)
Caullery and Mesnil (1914), C. R. Soc. Biol., 77:516-20.
Host: *Polydora ciliata.* (Polych.)

Polyrhabdina brasili
Caullery and Mesnil (1914) C. R. Soc. Biol., 77:516-20.
Spor. ovoidal, l. 200μ. Epim. like type, spines shorter.
Host: *Spio martinensis.* (Polych.)

Polyrhabdina pygospionis
Caullery and Mesnil (1914) C. R. Soc. Biol., 77:516-20.
Host: *Pygospionis seticornis.* (Polych.)

Family CEPHALOIDOPHORIDAE Kamm 1922 (this paper)
Characters of the type genus
Genus *Cephaloidophora* Mawrodiadi 1908

(=*Frenzelina* Léger and Duboscq 1907, preocc. See Arch. zool. exper., 46:lix-lxx.) Sporonts biassociative, no epimerite, cyst dehiscence by simple rupture, spores ovoidal with equatorial line. Development intracellular. Parasites of Crustacea.

Cephaloidophora maculata
Léger and Duboscq (1911) Arch. zool. exper., 46:lix-lxx.
Spor. ovoidal, max. l. 80μ. Nucl. spher. cysts spher. 100μ, spores spher. 4μ.
Host: *Gammarus marinus.* (Crust.) Taken at Roscoff, France.

Cephaloidophora talitri
Mercier (1912) C. R. Soc. Biol., 72:38-9.
Spor. ovoidal, average l. 40μ, nucl. spher.
Host: *Talitrus saltator.* (Crust.) Taken at Roscoff, France.

Cephaloidophora (=*Frenzelina*) *delphinia*[4]
Watson (1916) Jour. Parasit., 2:129-35.
Spor. ovoidal, largest spor. 115$\mu\times$64μ. LP:TL::1:4; WP:WD::1:1.5.[4]
Nucl. spher. Cysts spher. 80μ.
Host: *Talorchestia longicornis.* (Crust.) Taken at Cold Spring Harbor, L. I.

Cephaloidophora (=*Frenzelina*) *olivia*
Watson (1916) Jour. Parasit., 2:129-35.
Spor. ellipsoidal, largest 118$\mu\times$36μ. LP:TL::1:5; WP:WD::1:1.3.

[4] The ratios of length of protomerite to total length of sporont and width of protomerite to width of deutomerite are given for average individuals. These ratios will be abbreviated as above subsequently. Many recurring words will also be abbreviated from now on.

Cysts spher., 60μ.
Host: *Libinia dubia.* (Crust.) Taken at Cold Spring Harbor, L. I.

Cephaloidophora (=*Frenzelina*) *nigrofusca*
Watson (1916) Jour. Parasit., 2:129-35.
Spor. ovoidal, largest 125μ×75μ. LP:TL::1:4; WP:WD::1:1.5. Nucl. spher.
Hosts: *Uca pugnax*, *U. pugilator.* (Crust.) Taken at Cold Spring Harbor, L. I.

Cephaloidophora (=*Frenzelina*) *ampelisca*
Nowlin and Smith (1917) Jour Parasit., 4:83-88.
Spor. 62μ×15μ. Chromidial body in protomerite.
Host: *Ampelisca spinipes.* (Crust.) Taken at Woods Hole, Mass.

Family STENOPHORIDAE Léger and Duboscq 1904. *Spor. solitary,* Intracellular development. Dehiscence by simple rupture, spores ovoidal with equatorial line. Epimente absent or rudimentary. Parasites of Diplopoda.

Genus *Stenophora* Labbé 1899
With the characters of the family

Stenophora elongata
Ellis (1912) Zool. Anz., 39:685-6.
Spor. elongate-cylindr., max. l. 390μ. LP:TL::1:20; WP:WD::1:1 to 1:1.6. Prot. pentagonal.
Host: *Orthomorpha coarctata.* (Dipl.) Taken at Quirigua, Guatemala.

Stenophora cockerellae
Ellis (1912) Zool. Anz., 39:681-5.
Spor. elongate-cylindr., max. l. 850μ. LP:TL::1:15; WP:WD::1:1.7.
Host: *Parajulus* sp. (Dipl.) Taken at Quirigua, Guatemala.

Stenophora robusta
Ellis (1912) Zool. Anz., 40:8-11.
Spor. short, avg. l. 140-180μ, w. 67μ. LP:TL::1:8; WP:WD::1:2.5.
Hosts: *Parajulus venustus*; *Orthomorpha gracilis*; *O.* sp. (Dilp.) Taken at Boulder, Col.

Stenophora impressa
Watson (1915) Jour. Parasit., 2:29; (1916) Ill. Biol. Monogr., 2:280.
Spor. ellipsoidal, largest 375μ×48μ. LP:TL::1:12; WP:WD::1:2.3.
Cysts spher. 160μ.
Host: *Parajulus impressus.* (Dipl.) Taken at Urbana, Ill.

Stenophora diplocorpa
Watson (1915) Jour. Parasit., 2:29; (1916) Ill. Biol. Monogr., 2:284.

Spor. elongate-cylindr., constricted in mid-deut. LP:TL::1:20; WP:WD:: 1:2.
Host: *Euryurus erythropygus*. (Dipl.) Taken at Urbana, Ill.

Stenophora lactaria

Watson (1915) Jour. Parasit., 2:29; (1916) Ill. Biol. Monogr., 2:282.
Spor. elongate-ellips , largest 480$\mu \times 39\mu$ LP:TL::1:12; WP:WD::1:1 2.
Cysts spher. 170μ.
Host: *Callipus lactarius* (Dipl.) Taken at Urbana, Ill.

Stenophora caudata (=*Spirosoma caud.*)

Ishii (1915) Ann. zool. japon., 9:7-9.
Spor. tadpole-like in shape, posterior half reduced to cylindrical 'tail' knobbed at end and spirally striated. Max. l. 400μ, max. w. 100μ. LP:TL::1:12. Prot. papillate at apex.
Host: *Fontaneria coarctata* Pocock. (Dipl.) Taken in Gifu, Japan.

(The new genus *Spirosoma* Ishii is named from the spiral deutomerite, none of the generic characters—epimerite, cystdehiscence, spores—being found. From the few positive characters—shape of sporont, protomerite, and diplopod host—it appears to belong to *Stenophora*. The author's specimens were described from alcoholic specimens only.)

Stenophora cunhai

Pinto (1918) Brazil-Medico; (1919) Contribuição ao estudo das Gregarinas, Rio de Janeiro, 116 pp., 6 pl.
Spor. elongate-cylindr., prot. sub-spher., largest spor. 250$\mu \times 40\mu$. LP:TL:: 1:5; WP:WD::1:1. Nucl. spher., in post. part of deut.
Host: *Rhinocricus pugio*. (Dipl.) Taken at Rio de Janeiro, Brazil.

Stenophora lutzi

Pinto (1918) Brazil-Medico; (1919) Contribuição ao estudo das Gregarinas, Rio de Janeiro, 116 pp., 6 pl.
Spor. elongate-cylindr. prot. cylindr. with constriction below middle. Largest spor. 210$\mu \times 15\mu$. LP:TL::1:7.5; WP:WD::1:1.2. Nucl. small, spher.
Host: *Rhinocricus* sp. (Dipl.) Taken at Rio de Janeiro, Brazil

Stenophora cruzi

Pinto (1918) Brazil-Medico; (1919) Contribuição ao estudo das Gregarinas, Rio de Janeiro, 116 pp., 6 pl.
Spor. elongate-cylindr. conical posteriorly, largest spor. 400$\mu \times 30\mu$. LP:TL::1:13; WP:WD::1:2. Prot. a truncate cone. Nucl. unknown.
Host: *Rhinocricus* sp (Dipl.) Taken at Rio de Janeiro, Brazil.

Stephora viannai

Pinto (1918) Brazil-Medico; (1919) Contribuição ao estudo das Gregarinas, Rio de Janiero, 116 pp., 6 pl.

Spor. stout-cylindr., bluntly conical posteriorly, largest spor. 1000$\mu \times$150μ. LP:TL::1:16; WP:WD::1:2. Nucl. elongate-cylindr.

Host: *Rhinocricus* sp. (Dipl.) Taken at Rio de Janeiro, Brazil.

Stenophora umbilicata

Pinto (1918) Brazil-Medico; (1919) Contribuição ao estudo das Gregarinas, Rio de Janeiro, 116 pp., 6 pl.

Spor. stout-bodied, ovoidal, prot. small. broad, flat. 320$\mu \times$150μ. LP:TL:: 1:6; WP:WD::1:3.7. Nucl. spher.

Host: *Rhinocricus* sp. (Dipl.) Taken at Rio de Janeiro, Brazil.

Stenophora tenuicollis

Pinto (1918) Brazil-Medico; (1919) Contribuição ao estudo das Gregarinas, Rio de Janeiro, 116 pp., 6pl.

Spor. elongated globe-shaped in ant. fourth of deut. constricted to a 'wasp-waist' and widening gradually toward post. end, end broadly-rounded, prot. elongate-conical. Nucl. small, spher. Sporont 400$\mu \times$50μ.

Host: *Rhinocricus* sp. (Dipl.) Taken at Manguinhos, Rio de Janeiro, Brazil.

Genus *Fonsecaia* Pinto (1918)

Brazil-Medico; (1919) Contribuição ao estudo das Gregarinas, Rio de Janeiro, 116 pp., 6 pl. Like *Stenophora* except spores elongate-ellipsoidal, no endospore. Epimerite simple, without protoplasm. (The differentiation of this genus from *Stenophora* is not convincing.)

Fonsecaia polymorpha. Type species

Pinto (1918) Brazil-Medico; (1919) Contribuição ao estudo das Gregarinas, Rio de Janeiro, 116 pp., 6 pl.

Spor. 170$\mu \times$80μ. Broadly ovoidal, prot. small, conical. LP:TL::1:11.3; WP:WD::1:4.4. Nucl. spher. Spores ovoidal 18$\mu \times$8μ.

Host: *Orthomorpha gracilis*. (Dipl.) Taken at Rio de Janeiro, Brazil.

Family GREGARINIDAE Labbé 1899

Epimerite symmetrical. Sporonts associative or solitary. Cysts with or without spore-ducts.

Genus *Gregarina* Dufour 1828

Biassociative in sporont stage. Epimerite globular or cylindrical. Spores regular. Cysts with spore-ducts.

Gregarina ctenocephalus (=*G. ctenocephalus canis*)

Ross (1909) Ann. Trop. Med. Par., 2:359-63.

Spor. spherical, no dimensions given. Epimerite pyriform, spores barrel-shaped.

Host: *Ctenocephalus serraticeps* (Acarinidae.) Taken at Port Said, Egypt. Omitted from Sokolow's List.)

Gregarina erecta

Wellmer (1911) Schr. Physik. Ges. Königsbg., 52:115-6.

Spor. elongate-cylindrical, largest spor. 730μ×60μ. LP:TL::1:5; WP:WD ::1:1. Nucl. spher., cysts spher., 300μ, spores typical, 6.4μ×3.2μ.

Host: *Broscus cephalotes*. (Col.) Taken in East Prussia.

Gregarina ovoidea

Wellmer (1911) Schr. Physik. Ges. Königsbg., 52:117.

Spor. obese, max. l. 200μ. LP:TL::1:5; WP:WD::1:1.8. Nucl. spher. Cyst spher. 180μ.

Host: *Crypticus quisquilius*. (Col.) Taken in East Prussia.

Gregarina polyaulia

Wellmer (1911) Schr. Physik. Ges. Königsbg., 52:118-9.

Spor. cylindr., largest spor. 470μ×250μ. LP:TL::1:6; WP:WD::1:1.8. Cysts spher., 450μ, spores typical, 8.2μ×3.8μ.

Hosts: *Harpalus aeneus* and *H. ruficornis*. (Col.) Taken in East Prussia.

Gregarina rostrata

Wellmer (1911) Schr. Physik. Ges. Königsbg., 52:120-1.

Spor: elongate-ovoidal, largest spor. 200μ long. LP:TL::1:7; WP:WD:: 1:2. Nucl. spher., epimerite elongate-cylindrical. Cysts spher., 205μ, spores ovoidal, 5.6μ×3.2μ.

Host: *Lagria hirta*. (Col.) Taken in East Prussia.

Gregarina (Gigaductus) exiguus

Wellmer (1911) Schr. Physik. Ges. Königsbg., 52:121-2.

Spor. obese, max. length 75μ. LP:TL::1:4; WP:WD::1:2. Cysts spher., 35μ, one long spore-duct. Spores cylindr., 11.3μ×4.8μ.

Host: *Pterostichus niger*. (Col.) Taken in East Prussia. The genus *Gigaductus* has been dropped. See Watson (1916) Ill. Biol. Monogr., 2: 317, 389.

Gregarina guatemalensis

Ellis (1912) Zool. Anz., 39:687-8.

Spor. somewhat rectangular, max. l. 276μ. LP:TL::1:3; WP:WD::1:1.5. Nucl. spher.

Host: *Ninus interstitialis*. (Col.) Taken at Quirigua, Guatemala.

Gregarina consobrina

Ellis (1913) Trans. Amer. Micr. Soc., 32:267.

Spor. obese, average sporont 600μ l., 450μ, w. LP:TL::1:5; WP:WD:: 1:1.5. Cysts spher., 300μ. Sporeducts up to 1200μ in l. Spores 3.2μ×8μ.

Host: *Ceuthophilus valgus*. (Orth.) Taken near Boulder, Colo.

Gregarina grisea

Ellis (1913) Zool. Anz., 42:200.
Spor. ellipsoidal, max. l. 540μ. LP:TL::1:4; WP:WD::1:1.1. Nucl. spher.
Host: *Tenebrio castaneus*. (Col.) Taken at New Orleans, La.

Gregarina longiducta

Ellis (1913) Zool. Anz., 43:78-82.
Spor. obese, associations avg. 800-900μ in l. LP:TL::1:3; WP:WD::1:1. Cysts spher., 560μ. Spores $3\mu \times 6.5\mu$.
Hosts: *Ceuthopilus latens*, *C. maculatus*. (Orth.) Taken at Douglas Lake, Mich.

Gregarina typographi

Fuchs (1915) Zool. Jahrb., Syst., 38:109-222.
Spor. stout-bodied, bluntly ovoidal. No measurements given. LP:TL:: about 1:3; WP:WD::1:1. Nucl. small, spher. Cysts spher., one large spore-duct. Spores $34 \times 22\mu$.
Host: *Ips typographus*. (Col.) Taken in Southern Germany.

Gregarina (=*Clepsidrina*) *hylobii*

Fuchs (1915) Zool. Jahrb., Syst., 38:109-222.
Spor. long-ellipsoidal, largest spor. $847\mu \times 304\mu$. Nucl. elongate-ellipsoidal, one elongate karyosome. Cysts ovoidal $420 \times 370\mu$, without hyalin envelope, spore-ducts numerous, spores rectangular with spine at each corner, $6 \times 4\mu$.
Host: *Hylobius abietes*. (Col.) Taken in Southern Germany.

Gregarina minuta

Ishii (1914) Ann. Zool. japon, 8:436-8; Watson (1916) Ill. Biol. Monogr. 2:343, 392, 409.
Spor. elongate-cylindr., assn. l. 118μ. LP:TL::1:9; WP:WD::1:1.7. Nucl. spher., cysts spher., 48μ.
Host: *Tribolium ferrugineum*. (Col.) Taken in Prov. of Izu, Japan.

Gregarina globosa

Watson (1915) Jour. Parasit. 2:31; (1916) Ill. Biol. Monogr., 2:401.
Spor. subglobose, $260\mu \times 180\mu$. LP:TL::1:8.6; WP:WD::1:2.4. Nucl. spher.
Host: *Coptotomus interrogatus*. (Col.) Taken at Urbana, Ill.

Gregarina monarchia

Watson (1915) Jour. Parasit., 2:31; (1916) Ill. Biol. Monogr., 2:400.
Spor. elongate-cylindr., largest spor. $570\mu \times 130\mu$. LP:TL::1:7; WP:WD:: 1:1.3.
Host: *Pterostichus stygicus*. (Col.) Taken at Urbana, Ill.

Gregarina barbarara

Watson (1915) Jour. Parasit., 2:31; (1916) Ill. Biol. Monogr., 2:394. Spor. ovoidal, largest spor. 145$\mu\times$90μ. LP:TL::1:6; WP:WD::1:2. Nucl. small, spher.

Host: *Coccinella* sp. (Col.) Taken at Oyster Bay, L. I.

Gregarina katherina

Watson (1915) Jour. Parasit., 2:31; (1916) Ill. Biol. Monogr., 2:392. Spor. ellipsoidal, largest spor. 78$\mu\times$35μ. LP:TL::1:7; WP:WD::1:1.7. Nucl. spher.

Host: *Coccinella novemnotata.* (Col.) Taken at Oyster Bay, L. I.

Gregarina intestinalis

Watson (1915) Jour. Parasit., 2:32; (1916) Ill. Biol. Monogr., 2:399. Spor. broadly ellipsoidal, largest spor. 160$\mu\times$80μ. LP:TL::1:5; WP:WD:: 1:2.

Host: *Pterostichus stygicus.* (Col.) Taken at Urbana, Ill.

Gregarina gracilis

Watson (1915) Jour. Parasit., 2:32; (1916) Ill. Biol. Monogr., 2:398. Spor. elongate-ellipsoidal, largest spor. 190$\mu\times$80μ. LP:TL::1:8; WP:WD:: 1:2. Nucl. spher. cysts spher. 90μ.

Host: Larva of Elateridae. (Col.) Taken at Urbana, Ill.

Gregarina tenebrionella

Watson (1915) Jour. Parasit., 2:32; (1916) Ill. Biol. Monogr., 2:397. Spor. sub-globose, largest spor. 70$\mu\times$42μ. LP:TL::1:4; WP:WD::1:1.7. Nucl. spher.

Host: Larva of Tenebrionidae. (Col.) Taken at Urbana, Ill.

Gregarina fragilis

Watson (1915) Jour. Parasit., 2:32; (1916) Ill. Biol. Monogr., 2:395. Spor. ellipsoidal, largest spor. 110$\mu\times$60μ. LP:TL::1:5; WP:WD::1:2. Nucl. spher.

Host: *Coccinella* sp. (Col.) Taken at Urbana, Ill.

Gregarina nigra

Watson (1915) Jour. Parasit., 2:33; (1916) Ill. Biol. Monogr., 2:326. Spor. cylindrical, largest spor. 530$\mu\times$180μ. LP:TL::1:4; WP:WD:: 1:1.4. Nucl. spher.

Hosts: *Melanoplus femur-rubrum, M. differentialis, Encoptolophus sordidus.* (Orth.) Taken at Urbana, Ill.

Gregarina galliveri

Watson (1915) Jour. Parasit., 2:33; (1916) Ill. Biol. Monogr., 2:321.

Spor. 300μ×180μ. LP:TL::1:5; WP:WD::1:1. Prot. flat, broad, deut. widest in post. half. Nucl. spher. Cysts spher., 350μ.
Host: *Gryllus abbreviatus*. (Orth.) Taken at Oyster Bay, L. I.

Gregarina stygia

Watson (1915) Jour. Parasit., 2:33; (1916) Ill. Biol. Monogr., 2:324.
Spor. obese, largest 180μ×100μ. LP:TL::1:6; WP:WD::1:1.6. Nucl. spher., cysts spher. 150μ.
Host: *Ceuthophilus stygius*. (Orth.) Taken at Cold Spring Harbor, L. I.

Gregarina illinensis

Watson (1915) Jour. Parasit., 2:34; (1916) Ill. Biol. Monogr., 2:318.
Spor. elongate-cylindr., largest spor. 550μ×180μ. LP:TL::1:5; WP:WD:: 1:1.5. Nucl. small, spher.
Host: *Ischnoptera pennsylvanica*. (Orth.) Taken at Urbana, Ill.

Gregarina platyni

Watson (1916) Ill. Biol. Monogr., 2:402.
Spor. elongate-cylindr., max. l. 610μ. LP:TL::1:4; WP:WD::1:1. Prot. constricted in middle. Nucl. spher.
Host: *Platynus ruficollis*. (Col.) Taken at Oyster Bay, L. I.

Gregarina udeopsyllae

Watson (1916) Ill. Biol. Monogr., 2:327.
Spor. obese, largest 310μ×200μ. LP:TL::1:5; WP:WD::1:1.5.
Host: *Udeopsylla nigra*. (Orth.) Taken at Urbana, Ill.

Gregarina neglecta

Watson (1916) Jour. Parasit., 3:65-75.
Spor. ovoidal, largest spor. 500μ×230μ. LP:TL::1:6; WP:WD::1:1.5. Cysts spher., 300μ.
Host: *Ceuthophilus neglectus* (Orth.) Taken at Oyster Bay, L. I.

Gregarina platydema

Kamm (1918) Jour. Parasit., 4:159-63.
Spor. cylindr, slender, largest spor. 1210μ×150μ. LP:TL::1:12; WP:WD:: 1:1.5. Nucl. spher. Epim. a simple cone.
Host: *Platydema excavatum*. (Col.) Taken at Urbana, Ill.

Gregarina diabrotica

Kamm (1918) Jour. Parasit., 4:159-63.
Spor. elongate-cylindr. largest spor. 270μ×105μ. LP:TL::1:3.5; WP:WD:: 1:1.6. Nucl. spher. Epim. a sessile knob.
Host: *Diabrotica vittata*. (Col.) Taken at Urbana, Ill.

Gregarina watsoni

Pinto (1918) Brazil-Medico; (1919) Contribuição ao estudo das Gregarinas, Rio de Janeiro, 116, pp.; 6 pl.

Spor. elongate-ovoidal, largest spor. 350$\mu\times$152μ. LP:TL::1:7; WP:WD::1:1.5. Nucl. spher. Epim. globular.

Host: *Omoplata normalis.* (Col.) Taken at Nictheroy, Brazil.

Gregarina chagasi

Pinto (1918) Brazil-Medico; (1919) Contribuição ao estudo das Gregarinas, Rio de Janeiro, 116 pp., 6 pl.

Spor. sub-globular to cylindrical. Largest spor. 130$\mu\times$50μ. LP:TL::1:3.6; WP:WD::1:1.5. Nucl. spher. Cysts ovoidal.

Host: *Conocephalus frater.* (Orth.) Taken at Manguinhos, Brazil.

Gregarina aragãoi

Pinto (1918) Brazil-Medico; (1919) Contribuição ao estudo das Gregarinas, Rio de Janeiro, 116 pp., 6 pl.

Spor. elongate-ovoidal, max. l. 170μ, max. w. 70μ. LP:TL::1:5.7; WP:WD::1:1.7. Nucl. spher. Epim. a small papilla. Cysts subspherical.

Host: *Systena* sp. (Col.) Taken at Manguinhos, Brazil.

Gregarina sp.

Wellmer (1911) Schr. Physik. Ges. Königsbg., 52:146.

Host: *Sminthurus fuscus.* (Thysan.) Taken in East Prussia.

Gregarina sp.

Wellmer (1911) Schr. Physik. Ges. Königsbg., 52:148.

Host: *Oribata geniculata.* (Arachn.) Taken in East Prussia.

Genus *Hirmocystis* Labbé 1899

Associations of two to twelve or more. Epimerite a small papilla. Cysts dehisce by simple rupture. Spores ovoidal.

Hirmocystis harpali

Watson (1916) Ill. Biol. Monogr., 2:378.

Spor. elongate, largest 500$\mu\times$80μ. LP:TL::1:7; WP:WD::1:1.2. Maximum of four in a chain. Nucl. spher. Epim. large and spherical.

Host: *Harpalus pennsylvanicus erythropus.* (Col.) Taken at Urbana, Ill.

Genus *Uradiophora* Mercier 1912

Arch. zool. expér., (5) 10:198. Sporonts associative, cysts without spore-ducts. Spores spherical or sub-spherical with equatorial line, extruded in chains. Epimerite an elongated papilla. Deut. with small appendix.

Uradiophora cuénoti (=*Cephaloidophora cuénoti*) Type species.

Mercier (1911) C. R. Soc. Biol., 71:51-3; (1912) Arch. zool. expér., (5) 10:177-202.

Spor. associated in chains of from 2 to 4 individuals, very elongate, max. l. spor. 700μ. Nucl. spher. Epim. an elongated papilla. Deut. with small atrophied appendix. Cysts ovoidal, 44μ in l., spores 4μ.
Host: *Atyaëphyra Desmaresti*. (Crust.) Taken at Nancy, France.

Genus *Pyxinoides* Trégouboff 1912

Arch. zool. expér., (5) 10:liii-lxi. Sporonts in twos, development extracellular, epimerite a slightly stalked globular papilla with 16 longitudinal furrows, with small conical papilla at apex.
Pyxinoides balani. Type species
Trégouboff (1912) Arch. zool. expér., (5) 10:liii-lxi.
Max. l. primite 130μ, satellite 60μ. Nucl. spher.
Hosts: *Balanus amphitrite*, *B. eburneus*. (Crust.) Taken at Cette, France.

Genus *Leidyana* Watson 1915

Jour. Parasit., 2:35. Sporonts solitary, epimerite a small sessile knob, dehiscence by spore-ducts, spores in chains, dolioform.
Leidyana (=*Stenophora*) *erratica*. Type species.
Crawley (1903) Proc. Acad. Nat. Sci., Phila., 55:45.
Watson (1916) Ill. Biol. Monogr., 2:328-30.

Leidyana tinei
Keilin (1918) Parasit., 10:406-10.
Spor. long-ellipsoidal, max. l. 300μ, w. 85μ. LP:TL::1:1.5; WP:WD::1:1.7. Cysts spher. 110μ, spores barrel-sh., 7μ long.
Host: *Endrosis fenestrella*. (Lepid.) Taken at Cambridge, Eng.

Genus *Protomagalhãensia* Pinto 1918, Brazil-Medico

Spores barrel-shaped with spine at each corner, sporonts attenuated, several individuals in an association, often attached laterally. Myonemes prominent.
Protomagalhãensia (=*Gregarina*) *serpentula*. Type species.
Magalhães (1900) Arch. parasit., 3:34-69; Pinto (1918) Brazil-Medico.

Family DIDYMOPHYIDAE Léger 1892

Associations of two or three individuals. None-septate in satellites.

Genus *Didymophyes* Stein 1848

Epimerite a small pointed papilla. Cyst dehiscence by simple rupture. Spores ellipsoidal.
Didymophyes (=*Gregarina*) *minuta*
Ishii (1914) Ann. Zool. japon., 8:435-41.
Watson (1916) Ill. Biol. Monogr., 2:343.
Sporonts elongate, 188μ×26μ. Ratio LP:TL::1:23; WP:WD::1:1.5. Nucleus spherical. Cyst and spores unknown.
Host: *Tribolium ferrugineum*. (Col.) Taken in Prov. of Izu, Japan.

Family ACTINOCEPHALIDAE Léger 1892

Sporonts solitary, epimerites varied, simple rupture of cysts.

Genus *Actinocephalus* Stein 1848

Epimerite with many upwardly-directed spines, spores biconical.

Actinocephalus permagnus (?*A.* sp. Pfeiffer 1893; *A. stelliformis* Wasielewski 1896)

Wellmer (1911) Schr. Physik. Ges. Königsbg., 52:

Spor. elongate, max. 1. 1.3 mm. LP:TL::1:17; WP:WD::1.1:1. Nucl. ellipsoidal, cysts nearly spher., 750μ. Spores diamond-shaped, 7.6.$\mu\times$5μ.

Host: *Procrustes coriaceus.* (Col.) Taken in East Prussia.

Actinocephalus parvus

Wellmer (1911) Schr. Physik. Ges. Königsbg., 52:?.

Spor. ovoidal, largest 140$\mu\times$75μ. LP:TL::1:5; WP:WD::1:1.3. Nucl. ovoidal. Epim. a corona of digitiforn processes upon a short neck.

Hosts: *Ceratophyllus fringillae, C. gallinae* larv. (Dipt.) Taken in East Prussia.

Actinocephalus echinatus

Wellmer (1911) Schr. Physik. Ges. Königsbg., 52:?

Spor. cylindro-conical, largest 400μ in l. LP:TL::1:5; WP:WD::1.1:1. Cysts spher., 330μ, spores biconical, 8$\mu\times$4.8μ.

Hosts: *Pterostichus niger, P. vulgaris.* (Col.) Taken in East Prussia.

Actinocephalus zophus (=*Stephanophora zopha* Ellis (1913) Zool. Anz., 42:200-2).

Ellis (1913) Trans. Amer. Micr. Soc., 32:278.

Spor. elongate-cylindr., max. l. 1600μ. LP:TL::1:12; WP:WD::1:1.7. Epim. persistent, stout-necked, constricted at base, and terminating in corona of 9 or more small digitiform processes.

Hosts: *Nyctotheres barbarata* (*N. barbata*), *Alobates pennsylvanicus.* (Col.) Taken at New Orleans, La.

Actinocephalus brachydactylus

Ellis (1913) Trans. Amer. Micr. Soc., 32:279.

Spor. elongate-ovoidal, l. 500μ. LP:TL::1:4; WP:WD::1:1.

Host: Nymphs of Aeschna sp. (Neur.) Taken at Douglas Lake, Mich.

Actinocephalus crassus (=*Stephanophora crassa* Ellis (1912) Zool. Anz., 39:688-9).

Ellis (1913) Trans. Amer. Micr. Soc., 32:278.

Avg. spor. 50μ—60μ in l. LP:TL::1:3.5; WP:WD::1:1.5. Nucl. spher.

Host: *Leptochirus edax.* (Col.) Taken at Quirigua, Guatemala.

Actinocephalus gimbeli (=*Stenophora gimbeli* Ellis (1913) Zool. Anz., 41:464.) Watson (1916) Ill. Biol. Monogr., 2:353.

Spor. obese, l. 500μ. LP:TL::1:5; WP:WD::1:1.2.
Host: *Harpalus pennsylvanicus*. (Col.) Taken at Vincennes, Ind.

Genus *Pyxinia* Hammerschmidt 1838

Epimerite a flat crenulate crateriform disc with central style. Spores biconical.

Pyxinia bulbifera

Watson (1916) Jour. Parasit., 3:65-75.
Spor. long, slender, longest spor. 850μ×160μ. LP:TL::1:5. WP:WD:: 1:1.3. Epim. typical, 60μ–100μ l. Nucl. spher.
Host: *Dermestes lardarius*. (Col.) Taken at Oyster Bay, L. I.

Genus *Amphorocephalus* Ellis 1913

Zool. Anz., 41:462. Epim. dilated in middle, terminating in a concave disc peripherally fluted at ant. end. Prot. constricted across middle. Spores not known.

Amphorocephalus amphorellus. Type species.

Ellis (1913) Zool. Anz., 41:463-4; Trans. Amer. Micr. Soc., 32:276-7.
Spor. elongate, l. 500μ–970μ. LP:TL::1:17; WP:WD::1:2.
Host: *Scolopendra heros*. (Chil.) Taken at Boulder, Col.

Genus *Bothriopsis* Schneider 1875

Epimerite with long slender filaments. Prot. very large. Spores biconical.

Bothriopsis (=*Legeria*) *terpsichorella*

Ellis (1913) Trans. Amer. Micr. Soc., 32:276; Watson (1916) Ill. Biol. Monogr., 2:356.
Prot. of spor. equal to or longer than deut. Avg. l. 720μ, w. 145μ. LP:TL:: 1.5:1; WP:WD::1.3:1.
Host: *Hydrophilus* sp. (Col.) Taken at Douglas Lake, Mich.

Bothriopsis claviformis

Pinto (1918) Brazil Medico; (1919) Contribuição ao estudo das Gregarinas, Rio de Janeiro, 116 pp., 6 pl.
Spor. elongate-triangular, widest at ant. end, bluntly acuminate. LP:TL:: 1:7; WP:WD::1.4 : 1.
Host: *Aeschnida* larva. (Odon.) Taken at Manguinhos, Brazil.

Bothriopsis oswaldocruzi

Hasselmann (1918) Brazil-Medico, Nov. 2, 1918.

Genus *Stylocystis* Léger 1899

Epimerite a sharp recurved cone. Spores biconical.

Stylocystis ensiferus (=*Stylocephalus en.* Ellis 1912 Zool. Anz., 39:686)

Ellis (1913) Trans. Amer. Micr. Soc., 32:274.
Avg. l. spor. 40-65μ. LP:TL::1:2.5; WP:WD::1:1.2.
Host: *Leptochirus edax*. (Col.) Taken at Quirigua, Guatemala.

Genus *Steinina* Léger and Duboscq 1904

Epimerite a short digitiform process changing into a flat button. Spores biconical.

Steinina rotundata

Ashworth and Rettie (1912) Proc. Roy. Soc. Lond., B 86:31.

Spor. 180μ long, 80μ wide. Epim. sometimes a blunt cone with central style, again a saucer-shaped disc with crenulate periphery. Spor. ovoidal, nucl. spher. Cysts spher. 185μ, dehiscing in int. of host, spores ovoidal, 12$\mu\times$7μ.

Hosts: *Ceratophyllus styx*, *C. farreni*, *C. gallinae*. (Dipt.) Taken near Edinburgh, Scotland.

Steinina obconica

Ishii (1914) Ann. zool. japon., 8:439.

Spor. ovoidal, largest 148$\mu\times$80μ. LP:TL::1:5; WP:WD::1:1. Epim. a minuted style. Prot. compressed ant.-post. Nucl. spher. Cysts ovoidal.

Host: *Tribolium ferrugineum*. (Col.) Taken in Prov. of Izu, Japan.

Steinina rotunda

Watson (1915) Jour. Parasit., 2:32; (1916) Ill. Biol. Monogr., 2:364.

Spor. globose, largest 250$\mu\times$130μ. LP:TL::1:2.3; WP:WD::1:1.1. Epim. spher.

Host: *Amara angustata*. (Col.) Taken at St. Joseph, Ill.

Steinina harpali

Watson (1916) Ill. Biol. Monogr., 2:365.

Coelomic. Spor. small, obese, largest spor. 200$\mu\times$100μ. LP:TL::1:4; WP:WD::1:1.3. Epim. a short cone changing into a sphere then cup-shaped. Nucl. small, spher. Cysts spher. 12μ.

Host: *Harpalus pennsylvanicus longior*. (Col.) Taken at Urbana, Ill.

Family ACANTHOSPORIDAE Léger 1892

Spor. solitary, epim. varied. Dehiscence by simple rupture, spores with equatorial and polar spines.

Genus *Corycella* Léger 1892

Epim. globular with 8 large recurved hooks, spores biconical, 4 spines at each pole.

Corycella orthomorpha

Hasselmann (1918) Brazil-Medico, Oct. 5, 1918.

Genus *Prismatospora* Ellis 1914

Trans. Amer. Micr. Soc., 33:215. Spores hexagonal, truncate at ends with one row of long spines at each pole. Epim. subglobose with lateral recurved hooks.

Prismatospora evansi. Type species

Ellis (1914) Trans. Amer. Mic. Soc., 33:215.

Spor. 400μ in avg. l., broadly conical, LP:TL::1:3; WP:WD::1:1; Prot. broad, blunt, deut. tapering. Nucl. small, spher. Cysts subspher., 370μ, dehiscence by simple rupture, spores 11μ×5.8μ

Hosts: Nymphs of *Tramea lacerata* and *Sympetrum rubicundulum.* (Neur.) Taken at Douglas Lake, Mich.

Genus *Cometoides* Labbé 1899

Epim. a sphere with long slender filaments. Spores biconical with one polar and two equatorial rows of spines.

Cometoides sp.

Wellmer (1911) Schr. Physik. Ges. Königsbg., 52:138.

Spor. cylindro-conical. Max. 1. 360μ. LP:TL::1:5; WP:WD::1:1. Nucl. ellipsoidal. Epim. a flattened papilla with long filaments. Cysts spher. 160μ.

Host: *Carabus* sp. larva. (Col.) Taken in East Prussia.

Family STYLOCEPHALIDAE Ellis

New name for Stylorhynchidae Schneider 1886 preocc. Ellis (1912) Zool. Anz., 39:25. Spor. solitary, epim. varied, nucl. ovoidal, dehiscence by pseudocyst, spores irregular, in chains.

Genus *Stylocephalus* Ellis

New name for Stylorhynchus Stein 1848 preocc. Ellis (1912) Zool. Anz., 39:25. Epim. a papilla at end of a long slender neck. Cysts papillate, spores hat-shaped.

Stylocephalus giganteus

Ellis (1912) Zool. Anz., 39:25-7.

Spor. elongate, 1200-1800μ in 1. LP:TL::1:15; WP:WD::1:1. Cysts spher., 450μ. Spores 7×11μ.

Hosts: *Eleodes* sp.; *Asida opaca; Asida* sp.; *Eusattus* sp. (Col.) Taken at Boulder and Denver, Col.

Genus *Bulbocephalus* Watson 1916

Jour Parasit., 3:66. Epim. a dilated papilla in middle of rather long slender neck. Nucl. ellipsoidal.

Bulbocephalus wardi. Type species

Watson (1916) Jour. Parasit., 3:66.

Spor. stout, widest at shoulder, largest spor. 290μ×45μ. LP:TL::1:5; WP:WD::1:1. Epim. as above. Nucl. placed diagonally. Cysts and spores unknown.

Host: *Clerid* larva. (Col.) Taken at Oyster Bay, L. I.

Bulbocephalus elongatus

Watson (1916) Jour. Parasit., 3:66.

Spor. very long and slender, max. l. 600μ, w. 50μ. LP:TL::1:11; WP:WD::1:1. Epim. as above. Nucl. diagonally placed.

Host: *Cucujus* larva. (Col.) Taken at Oyster Bay, L. I.

Family DACTYLOPHORIDAE Léger 1892

Epimerite complex, sporonts solitary, cysts dehisce by lateral pseudocyst or simple rupture, spores elongate.

Genus *Nina* Grebnecki 1873

Protomerite two long lobes fused at one end, peripherally set with teeth and long slender filaments. Spores in chains.

Nina indicia

Merton (1911) Abh. Seneck. nat. Ges. Frankf., 34:119-26.

Spor. elongate, max. l. 1500μ. LP:TL::1:20; WP:WD::4:1. Prot. low, very broad, two long narrow parallel plates attached laterally, free at one end, each plate armed with a ridge of short sharp teeth. Nucl. spher.

Host: *Scolopendra subspinipes*. (Chil.) Taken at Heidelberg, Germ.

Nina (=*Pterocephalus*) *leitâodacunhai*

Hasselmann (1918) Brazil-Medico, Sept. 21, 1918.

Genus *Echinomera* Labbé 1899

Epimerite an eccentric cone with short digitiform processes. Dehiscence by simple rupture. Spores cyllindrical, in chains.

Echinomera (=*Gregarina*) *magalhaesi*[5]

Pinto (1918) Brazil-Medico; (1919) Contribuição ao estudo das Gregarinas, Rio de Janeiro, 116 pp., 6 pl.

Spor. elongate-conoidal, widest at shoulder. Largest spor. 300$\mu\times$70μ. LP:TL::1:4.3; WP:WD::1:1.1. Epim. a polymorphic eccentric cone. Nucl. ellipsoidal.

Host: *Scolopendra* sp. (Chil.) Taken at Rio de Janeiro, Brazil.

Genus *Seticephalus* Kamm 1922 (this paper)

A dense tuft of short, upwardly-directed brush-like bristles superimposed upon the broad, flat-topped protomerite, persistent. A chromidial body in protomerite. Parasitic in Chilopoda.

Seticephalus (=*Gregarina*) *elegans*. Type species.

Pinto (1918) Brazil-Medico; (1919) Contribuição ao estudo das Gregarinas, Rio de Janeiro, 116 pp., 6 pl.

Spor elongate-conoidal, acuminate, largest spor. 75$\mu\times$32μ. LP:TL::1:7.5; WP:WD::1:1.2. Prot. broad, flat, nucl, small ellipsoidal. Epim.

[5] This species was placed by the author in the Genus *Gregarina*.

short bristle-like filaments across whole ant. end of prot. Cyst and spores unknown.

Host: *Scolopendra* sp. (Chil.) Taken at Rio de Janeiro, Brazil.

(This species was placed by the author in the genus *Gregarina* but it is unlike any member of that genus or any hitherto described genus and is therefore made the type species of a new genus.)

Genera of Uncertain Position

Genus *Agrippina* Strickland 1912 Parasit., 5:108

Sporonts solitary, epim. a circular disc armed with peripheral digitiform processes, with short neck. Spores long-ovoidal.

Agrippina bona. Type species

Strickland (1912) Parasit., 5:108.

Spor. elongate, conical, avg. 1. 175μ. Nucl. ellipsoidal. Epim. as stated. Cysts spher. dehiscing by simple rupture. Spores 6.6$\mu \times 7\mu$.

Host: *Ceratophyllus fasciatus.* (Dipt.) Taken at Cambridge, England.

Genus *Metamera* Duke 1910

Q. J. Mic. Sci., 55:261-86. Epimerite subconical, apex eccentric, with corona of numerous branched digitiform appendages. Cysts dehisce by simple rupture. Sporonts solitary.

Metamera schubergi. Type species

Duke (1910) Q. J. Mic. Sci., 55:261-86.

Spor. 150$\mu \times 45\mu$. Deut. with one to three septa posterior to nucleus. Cysts spher., spores ovoidal, 9$\mu \times 7\mu$.

Hosts: *Glossophonia complanata, Hemiclepsis marginata.* (Annelida.) Taken at Heidelberg, Germ. and Cambridge, Eng.

(This species was left out of Sokolow's Synopsis (1911).)

Genus *Ganymedes* Huxley 1910

Q. J. Mic. Sci., 55:155-75. Non-septate, with motile extensile fixation-organ, cupped posterior end for association, nucleus large, spherical. Inhabit intestine and liver of host.

Ganymedes anasidis

Huxley (1910) Q. J. Mic. Sci., 55:155-75.

Characters of the genus. Avg. 1. 250-300μ, w. 17-20μ. Spor. elongate-cylindrical.

Host: *Anaspides tasmaniae.* (Crust.) Taken in Tasmania.

(This species was omitted from Sokolow's List—1911.)

Species of Uncertain Position

Gregarina crassa

Ishii (1915) Ann. zool. japon., 8:438-9.

Spor. ovoidal, max. l. 242μ, w. 64μ. Nucl. spher. LP:TL::1:19; WP:WD::1:4.

Host: *Tribolium ferrugineum.* (Col.) Taken in Prov. of Izu, Japan. Prot. lacking in satellite. See Watson (1916) Ill. Biol. Monogr., 2:409.

Gregarina coptotomi

Watson (1916) Jour. Parasit., 2:406.

Spor. solitary, epim. and cysts unknown. Spor. 210μ l. LP:TL::1:7; WP:WD::1:2.3. Nucl. ellipsoidal.

Host: *Coptotomus interrogatus.* (Col.) Taken at Urbana, Ill.

Gregarina brasiliensis

Pinto (1918) Brazil-Medico; (1919). Contribuicao ao estudo dos Gregarinos Rio de Janeiro, 116 pp., 6 pl.

Spor. not associative, pyriform, acutely acuminate, largest spor. 92$\mu\times$35μ. LP:TL::1:2.4; WP:WD::1:1.1. Prot. ovo-cylindrical, nucl. ovoidal.

Host: *Scolopendra* sp. (Chil.) Taken at Rio de Janeiro, Brazil.

Gregarina légeri

Pinto (1918) Brazil-Medico; (1919). Contribuicao ao estudo dos Gregarinos Rio de Janeiro, 116 pp., 6 pl.

Spor. not associative, rectangular with bulbous post. extremity, largest spor. 290$\mu\times$80μ (at post. end of deut.) LP:TL::1:4.8; WP:WD::1:1. Prot. square, nucl. ellipsoidal, in dilated post. portion of deut.

Host: *Stylopyga americana.* (Orth.) Taken at Rio de Janeiro, Brazil.

Taeniocystis legeri

Cognetti de Martiis (1911) Arch. Protistenk., 23:247.

Spor. segmented in both prot. and deut., max. l. 1600μ. Up to 19 segments. Nucl. ovoidal. Epim., cysts, and spores unknown.

Host: *Kynotus Pittarellii* (Oligoch.) Taken at Moramanga, Madagascar.

This species is placed among the 'Uncertain Species' because the 'protomerite' is divided into three segments and the parasite is coelomic.

Miscellaneous

Unnamed gregarines:

Wellmer (1911) Schr. Physik. Ges. Königsbg., 52:46-7. From the following hosts: *Heledona agricola* (Col.), *Polyporus sulphureus* (Plathy.), *Tritoma quadripustulata* (Col.), *Cychrus rostratus* (Col.), *Scolopendrella* sp. (Chil.).

Unnamed Cometoides-like form:

Wellmer (1911) Schr. Physik. Ges. Königsbg., 52:46-7. Host: *Hydrophilus aterrimus.* (Col.).

Unnamed gregarines of several species:

Pantel (1913) La Cellule, 29 (1): 142-4. Host: *Forficula auricularia.* (Orth.)

Unnamed gregarine:
Buddington (1910) Science, 31:470. Host: *Balanus eburneus*. (Crust.)

Unnamed gregarines similar to *Leidyana tinei*:
Keilin (1918) Parasit., 10:406. Hosts: *Oecophora pseudospretella*, *Tinea pallescentella*. (Lepidopt.)

An Annotated List of Species in the Tribe Acephalina of the Suborder Eugregarinae

Genus *Monocystis* Stein 1848

Non-septate, irregular, motile sporonts, cysts with incomplete sporulation, spores navicular, octozoic. (All herein described are coelomic or inhabit seminal vesicles unless stated otherwise.)

Monocystis pareudrili
Cognetti de Martiis (1911) Arch. Protistenk., 23:216-40.
Spor. subspherical, max. diam. 60μ. Spores ovoidal, 10$\times$5μ.
Host: *Pareudrilus pallidus*. (Polych.) Taken in 'Equatorial Africa.'

Monocystis thamnodrili (=*M.* sp. Cognetti 1906)
Cognetti de Martiis (1911) Mem. R. Accad. Sci. Torino, 46: (2) 147-262.
Host: *Rhinodrilus* (=*Thamnodrilus*) *incertus*. (Polych.) Taken in Ecuador.

Monocystis rostrata
Muslow (1911) Arch. Protistenk., 22:20-55.
Sem. ves. Spor. spindle-shaped, cysts spher., spores spindle-shaped.
Host: *Lumbricus terrestris*. (Oligoch.) Taken in Munich.

Monocystis catenata (=partim. *M. herculea* Hesse 1909)
Muslow (1911) Arch. Protistenk., 22:51.
Spor. spher. 425μ, in chains. Cysts nearly spher. 500μ. Spores 14$\times$6μ.
Host: *Lumbricus terrestris*. (Oligoch.)

Monocystis minima
Konsuloff (1916) Arch. Protistenk., 36:353-61.
Spor. ovoidal, 42μ, spores ellipsoidal, 7μ long. Intestinal par.
Hosts: *Euchlanis dilatata*. (Rotif.); *Salpina mucronata* Ehrbg. (Rotif.)
Taken at Sofia.

Monocystis perforans
Pinto (1918) Brazil-Medico; (1919) Contribuição ao estudo das Gregarines, Rio de Janeiro, 113 pp., 6 pl.
Sem. ves. Spor. ovoidal to cylindr. in chains. 1200$\mu$$\times$800$\mu$. Nucl. ellipsoidal, cysts spher. spores 24$\times$7.5μ.
Host: *Glossoscolex wiengreeni*. (Ann.) Taken at Rio de Janeiro, Brazil.

Monocystis michaelseni
Hesse (1916) Tolosani Monit. Zool. ital , 27:217-22.

Monocystis sp.
Wellmer (1911) Schr. Physik. Ges. Königsb., 52:147. Coleomic.
Host: *Helophorus aquaticus.* (Col.) Taken in East Prussia.

Genus *Lithocystis* Giard 1876

Emend. Pixell-Goodrich (1915) Q. J. Mic. Sci., 61:81-104. Spor. elongate, very motile. Spores in rosettes, long ovoidal, truncate. Epispore a funnel at one end through which 8 sporozoites escape, other end a tubular tail.

Lithocystis foliacea
Pixell-Goodrich (1915) Q. J. Mic. Sci., 61:81-104.
Coelomic. Max. l. sporont 1.3 mm. Cysts spher. 600μ. Spores long-ovoidal, 24$\times$9μ, tail three times as long as spore, with leaf-like expansion, funnel at other end.
Host: *Echinocardium cordatum.* (Echinodermata.) Taken at Naples and Plymouth, Eng.

Lithocystis microspora
Pixell-Goodrich (1915) Q. J. Mic. Sci., 61:81-104.
Coelomic. Max. l. 1mm. Cysts spher. 300μ. Spores 13$\times$7μ, with tail two or three times as long, narrow, tapering.
Host: *Spatangus purpureus.* (Echinod.) Taken off Plymouth.

Genus *Urospora* Schneider 1875

Emend. Pixell-Goodrich (1915) Q. J. Mic. Sci., 61:81-104. Trophozoites coarsely granular, elongate. Female gamete with long flagellum, male non-motile. Cysts spher. 8 sporozoites which escape from one end of funnel-shaped epispore other end a filamentous tail.

Urospora synaptae (=*Syncystis syn.* Cuénot (1891) Rev. Biol. nord. Fr., 3:295.)
Cuénot (1912) Bull. sta. biol. Arcachon, Bord., 14:85.
One form spor. rotund, 300μ max. diam, other vermiform 500μ long, very motile. Cysts spher. 150μ, spores ovoidal, 20μ, one end cupped other a long filament. Coelomic and intestinal.
Host: *Synapta galliennei.* (Echinod.) Taken at Arcachon and Roscoff, Fr.

Urospora travisiae

Urospora ovalis
Mawrodiadi (1914) Varsava Univ. izv., No. 8, 1-164.

Urospora neapolitana

Pixell-Goodrich (1915) Q. J. Mic. Sci., 61:81-104. See also Q. J. Mic. Sci., 60:159-74.

Spor. 200-300μ l., 40μ w. Cysts spher. 100-200μ. Spores 12$\times$7μ, ovoidal, cupped at one end, tail twenty times as long as spore and tightly coiled at other end.

Host: *Echinocardium cordatum.* (Echinod.) Taken at Naples.

Urospora echinocardii

Pixell-Goodrich (1915) Q. J. Mic. Sci., 61:81-104.

Troph. and cysts same as *U. neapolitana.* Spores 19μ long, tails 6 or 7 times as long as spore, not tightly coiled.

Hosts: *Echinocardium* sp. and *Spatangus* sp. (Echinod.) Taken at Plymouth, Eng.

Genus *Gonospora* Schneider 1875. Emend

Pixell-Goodrich (1916) Q. J. Mic. Sci., 61:205-16. Polymorphic, nematoid, pyriform or ovoidal. Cysts spher., spores with funnel at one end refringent endo-spore which gives off processes supporting thick transparent ectospore and funnel at other.

Gonospora mercieri (= *Lithocystis mülleri* Giard 1886.)

Cuénot (1912) Bull. sta. biol. Arcachon, Bord., 14:88-90.

Spor. spher., max. diam. 160μ. Cysts 180μ in diam., spores 23μ long, ovoidal, no caudal filament. Intestinal par.

Host: *Synapta digitata.* (Echinod.) Taken at Arcachon, France.

Gonospora glycerae

Pixell-Goodrich (1916) Q. J. Mic. Sci., 61:205-16.

Coelom par., generally surrounded by host epithelium. Spor. 1 to 5 mm. long, widest near ant. end and tapering to blunt point. Nucl. spher. cysts spher. spores 10$\times$8μ. Refringent endospore with many supporting processes. Associations of spor. by 'ball-and-socket' dovetailing of ant. ends.

Host: *Glycera siphonostoma.* (Polych.) Taken at Naples.

Gonospora testiculi (= *Cystobia test.*)

Trégouboff (1916) C. R. Soc. Biol., 79:652-5; (1918) Arch. zool. expér., 57:471–509.

L. 250μ, elongate-ovoidal rounded at ant. end, pointed at post. end. Cysts 60-100μ in diam. Spores 8 to 10μ in l. Testicle par.

Host: *Cerithium vulgatum.* (Moll.) Taken at Villefranche-sur-Mer, France.

Gonospora intestinalis (= *Cystobia int.*)

Sokoloff (1914) Arch. Protistenk., 32:221-8.; Trégouboff, (1918) C. R. Soc. Biol., 79:652-55, Pixell-Goodrich (1916) Q. J. Mic. Sci., 61-205-16.

Intestinal par. Spor. elongate, max. l. 300μ. Cysts nearly spher. 300μ. Spores ovoidal, 10μ 1.
Host: *Glycera siphonstoma.* (Polych.) Taken at Naples.

Genus *Rhynchocystis* Hesse 1909

Spor. ovoidal to cylindr. ant. end conical. Spores biconical with like poles.

Rhynchyocystis hessei

Cognetti de Martiis (1911) Mem. R. Accad. Sci. Torino, 46:207-16.
Max. l. spor. 116μ, w. 88μ. Coelomic par. Spores 13$\times$2.5μ.
Host: *Pareudrilus pallidus.* (Polych.) Taken in 'Equatorial Africa.'

Rhynchocystis geoplanae

Fuhrmann (1916) Centrallbl. Bakt. Parasit., 77:482-5.
Parenchymatous and intestinal par. Largest spor. 280$\times$80μ. Nucl. large, spher. Cysts spher. 180μ. 'Pseudoepimerite' a rosette.
Hosts: *Geoplana backi, G. amagensis.* (Furh.) Taken in Columbia, S. A.

Genus *Diplocystis* Künstler 1887

Coelomic, associating early to form spherical masses. Spores spherical or oblong. Eight sporozoites.

Diplocystis phryganeae

Berg-von-Emme (1913) Arch. Protistenk., 28:43-51.
Spor. subspher. nucl. spher.
Host: *Phryganea grandis* (Neur.) Taken at Petrograd.

Genus *Lankesteria* Mingazzini 1891

Trophozoites spatulate, cysts spher., spores ovoidal.

Lankesteria sp.

Swarczewsky (1910) Festschr. Geburtst. R. Hertwig 1: 635-74; (1911) Arch. Protistenk., 22:236.
Intestinal par., encysted in parenchyme. Cysts spher. 200μ. Cyst walls dissolve and spores are carried to organs, set at liberty at death of host.
Hosts: *Planaria* sp. and *Sorocoelis* sp. (Plath.)

Lankesteria culicis

Stevenson and Wenyon Jour. Trop. Med. and Hyg., 18:196; Macfie (1917) Report of the Accra Lab. for 1916, London, pp. 67-75.
Host: *Stegomyia fasciata* larv. (Dipt.) Taken at Accra, Gold Coast, Africa.

Genus *Ancora* Labbé 1899

Anchor-shaped spor. two long lateral backwardly-directed prolongations from ant. end, body tapering to sharp point.

Ancora lutzi (?*A. sagittata* Leuckart Arch. Naturg., 26 (2):263)

Hasselmann (1918) Brazil-Medico, Aug. 10, 1918.
Host: *Capitella capitata* Fabr. (Ann.) Taken at Manguinhos, Rio de Janeiro, Brazil.

Uncertain Genus in the Acephalinae

Genus *Rhytidocystis* Henneguy 1908

Trophozoite stage intracellular, encystment solitary, two sporozoites in spore.

Rhytidocystis henneguyi

deBeauchamp (1912) C. R. Acad. Sci. Par., 154:1384; (1913) Arch. Protistenk., 31:138.

Spor. ellipsoidal, encystment solitary. Nucl. spher. Spores $12 \times 7\mu$, ovoidal, symmetrical. Epithelium and lumen of intest.

Host: *Ophelia neglecta.* (Polych.) Taken off Roscoff, France.

Uncertain Species in the Acephalinae

Unnamed sp.

Guenther (1914) Zool. Anz., 44:264-7.

Host: *Ficalbia dofleini.* larv. (Dipt.) Taken on Island of Ceylon. Habitat: Tracheae and coelom.

Unnamed sp.

Pixell-Goodrich (1916) Q. J. Micr. Sci., 61:205-16.

Max. l. 1.6 mm., w. 1 mm. Coelomic and attached to body or intest. walls. Nucl. large, ovoidal.

Host: *Glycera siphonostoma.* (Echinod.) Taken at Naples.

Two other unnamed sp. found by same author, and in same host as last.

An Annotated List of the Species in the Suborder Schizogregarinae

Tribe 1. MONOSPORA Léger and Duboscq 1908

Family 1. OPHRYOCYSTIDAE Léger and Duboscq 1908

Tribe 2. POLYSPORA Léger and Duboscq 1908

Family 2. SCHIZOCYSTIDAE Léger and Duboscq 1908

Genus *Schizocystis* Léger 1900

Schizonts extracellular, vermiform, multinucleate. Gametes ovoidal, pointed at one end. Cysts subspher. or ovoidal, spores octozoic, biconical.

Schizocystis spinigeri

Machado (1913) Mem. Inst. Oswaldo Cruz, Rio de Jan., 5:5-13.

Spor. slender, striated longitudinally, cysts ovoidal, spores ovoidal, pointed. Both sporogony and schizogony noted.

Host: *Spiniger* sp. (Hemipt.) Taken near Manguinhos, Rio de Janeiro, Brazil.

Family 3. SELENIDIIDAE Brazil 1907

Schizonts intracellular, multinucleate, at close of development. Gametocystes mobile, longitudinal myonemes. Parasitic in Polychaetes.

Genus *Selenidium* Giard 1884

Schizogony in the intracellular stage.

Selenidium cruzi

Faria, Cunha and Fonseca (1917) Brazil-Medico, 31:243; (1918) Mem. Osw. Cruz, 10:17.

Largest trophoz. 160μ×25μ, vermiform, slightly flattened, ant. end blunt with small pointed epimerite. Nucl. ellipsoidal.

Host: *Polydora socialis*. (Polych.) Taken at Rio de Janeiro, Brazil.

Selenidium mechnikovi

Léger and Duboscq (1917) Ann. Inst. Past., 31:69.

Intestinal par. Intra- and extra-cellular, schizozoites pyriform, 5μ.

Sporonts cucumber-shaped, 30-34μ, longit. striated, nucl. sub-spher.

Host: *Glossobalanus minutus*. (Enteropneusta.) Taken at Sainte-Jean-de-Luz, France.

Family 4. MEROGREGARINIDAE Porter 1908

Family 5. SPIROCYSTIDAE Léger and Duboscq 1915

Arch. Protistenk., 35:199-211. Mono-sporic and monozoic, schizogony and sporogony in same host.

Genus *Spirocystis* Léger and Duboscq

1911 Bull. Soc. zool. Fr., June, 1911.

Spirocystis nidula. Type species

Léger and Duboscq (1911) Bull. Soc. zool. Fr., June 1911; C. R. Soc. Biol., 76:296; Arch. Protistenk., 35:199.

Sporocyst ovoidal, 35μ long, rejected with excrement. Releases in its next host through a micropyle a single folded sporozoite 40μ long. Sporozoite gives rise to helix-shaped schizont found in somatic or visceralperitoneum. This becomes multinucleate of max. l. 35μ and gives rise to macro- and micro-gametes, the copulation of which produces the spore, found in the chloragogue cells.

Host: *Lumbricus variegatus*. (Oligoch.) Taken near Grenoble, France.

Tribe 3. OCTOSPOREA Keilin (1914) C. R. Soc. Biol., 76:768

With eight spores in the sporogonic cycle.

Family 6. CAULLERYELLIDAE Keilin (1914) C. R. Soc. Biol., 76:768.

Genus *Caulleryella* Keilin (1914) C. R. Soc. Biol., 76:768

Intestinal par. Schizogony extracellular, veg. nucleus gives rise to 16 merozoites. Each of the two sporonts in a cyst produces 8 gametes. These 16 conjugate by twos to form 8 spores which produce 8 sporozoites.

Caulleryella aphiochaetae. Type species

Keilin (1914) C. R. Soc. Biol., 76:768.

Veg. stage 22μ long, ovoidal, pointed at end, embedded in epithelium.

Nucl. divides four times, giving rise to 16 merozoites liberated and affix themselves to epithelium. Sporulation by twos with 16 gametes produced. Cysts and gametes spherical.
Host: *Aphiochaeta rufipes* larv. (Dipt.) Taken at Paris.

Caulleryella anophelis
Hesse (19?) C. R. Acad. Sci. Par., 166:569.
Spor. 35×30μ, syzygy in twos, cysts spher. 24μ to 32μ. Spores sub-spher. 12.5×11μ. Dehiscence of cyst in host intest.
Host: *Anopheles bifurcatus*. larv. (Dipt.) Taken in the Dauphine, France.

Family of Uncertain Position

Family 7. POROSPORIDAE Léger and Duboscq 1908

1915 C. R. Soc. Biol., 75:95-8. Many sporozoites from a sporoblast. No sporocyst.

Genus *Porospora* Schneider 1875

Epim. minute, button-like, spor. septate, usually solitary.
Porospora légeri
deBeauchamp (1910) C. R. Acad. Sci. Par., 151:997-9.
Spor. associative prot. of primite depressed at apex, satellite longer with no prot. 750μ×75μ. Cysts spher. from two sporonts. Intestinal par.
Host: *Eriphia spinifrons*. (Crust.) Taken at Saint-Jean-de-Luz, Fr.
(This species was omitted from Sokolow's List-1911.)

Porospora portunidarum Léger and Duboscq 1911 (=*Aggregata p.* Frenzel)
(1911) Arch. zool. exper., (5) 6:lix-lxx; (1913) C. R. Acad. Sci. Par., 156:1932; (1913) C. R. Soc. Biol., 75:95.

Porospora pisae
Léger and Duboscq (1911) Ann. Univ. Grenoble, 23:403; Trégouboff (1916) Arch. zool. exper., 55:xxxv-xlvii.
1 mm. long, eel-shaped. Encystment from one or two spor.
Host: *Pisa gibosii*. (Crust.) Taken at Cette and Villefranche-sur-Mer, Fr.

Porospora maraisi
Léger and Duboscq (1912) Ann. Univ. Grenoble, 23:399.
Host: *Portunus depurator*. (Crust.)

Porospora nephropsis
Léger and Duboscq (1915) C. R. Soc. Biol., 75:368-71.
Spor. elongate, ellipsoidal, blunt at ends, max. l. 240μ, max. w. 44μ. Nucl. spher. Solitary vermiform enigmatic individuals 1300×36 also present. Cysts 160μ in diam. Schizogonic spores 5 in diam.
Host: *Nephrops norvegicus*. (Crust.)

The classification of this family is uncertain because the sporonts are apparently typical cephaline Eugregarinae yet a schizogonic cycle exists. Minchin (1912) says 'The classification of the future will probably be one which divides all gregarines into Cephalina and Acephalina and distributes the schizogregarines amongst these two divisions.'

Genus of Uncertain Position

Genus *Selysina* Duboscq 1917 C. R. Acad. Sci., 164:?

Selysina perforans. Type species

Duboscq (1917) C. R. Acad. Sci., 164:? (1918) Arch. zool. exper., ?:1-53.

Host: *Stolonica socialis.* (Ascid.) Taken off Roscoff, France.

Unnamed gregarine

Strickland (1913) Jour. Morphol., 24:84.

Schizogonous. Pathogenic effect upon host. Inhabits various tissues. Cysts spher. 250μ.

Host: *Simulium bracteatum* larv. (Dipt.) Taken near Boston, Mass.

A species described as *Microtaeniella clymenellae* n.g., n. sp. from *Clymenella torquata* (Ann.) by Calkins (1915) Biol. Bull., 29:46 is regarded by the author as a colonial gregarine resembling the scolex and proglottids of the cestodes, each segment being nucleated. This polynucleate condition makes its inclusion in this group doubtful. Poche (Arch. Protistenk., 37:6) considers it identical with the genus *Haplozoon.*

List of Hosts With Their Gregarine Parasites

Host	Parasite
Platyhelminthes	
Geoplana backi	*Rhynchocystis geoplanae* Fuhrman
G. amagensis	*Rhynchocystis geoplanae*
Planaria sp.	*Lankesteria* sp. Swarczewsky
Polyporus sulphureus	Gregarine form, Wellmer
Sorocoelis sp.	*Lankesteria* sp. Swarczewsky
Annelida: Polychaeta	
Capitella capitata	*Ancora lutzi* Hasselmann
Clymenella torquata	*Microtaeniella clymenellae* Calkins
Glycera siphonostoma	*Gonospora glycerae* Pixell-Goodrich
Glycera siphonostoma	*Gonospora intestinalis* Pixell-Goodrich
Glycera siphonostoma	Three unnamed parasites Pixell-Goodrich
Ophelia neglecta	*Rhytidocystis henneguyi* deBeauchamp
Pareudrilus pallidus	*Monocystis pareudrili* Cognetti de Martiis
Pareudrilus pallidus	*Rhynchocystis hessei* Cognetti de Martiis
Polydora ciliata	*Polyrhabdina polydorae* Caullery and Mesnil
Polydora socialis	*Doliocystis* sp. Faria, Cunha and Fonseca
Polydora socialis	*Selenidium cruzi* Faria, Cunha and Fonseca
Pygospionis seticornis	*Polyrhabdina pygospionis* Caullery and Mesnil

Host	Parasite
Rhinodrilus incertus	*Monocystis thamnodrili* Cogn. de Martiis
Scolelepsis fuliginosa	*Polyrhabdina spionis* Caullery and Mesnil
Spio martinensis	*Polyrhabdina brasili* Caullery and Mesnil
Annelida: Oligochaeta	
Kynotus Pittarellii	*Taeniocystis legeri* Cogn. de Martiis
Lumbricus terrestris	*Monocystis rostrata* Muslow
Lumbricus terrestris	*Monocystis catenata* Muslow
Lumbricus variegatus	*Spirocystis nidula* Léger and Duboscq
Glossoscolex wiengreeni	*Monocystis perforans* Pinto
Annelida: Hirudinea	
Glossophonia complanata	*Metamera schubergi* Duke
Hemiclepsis marginata	*Metamera schubergi* Duke
Rotifera	
Euchlanis dilatata	*Monocystis minima* Konsuloff
Salpina mucronata	*Monocystis minima* Konsuloff
Echinodermata	
Echinocardium cordatum	*Lithocystis foliacea* Pixell-Goodrich
Echinocardium cordatum	*Urospora neapolitana* Pixell-Goodrich
Echinocardium sp.	*Urospora echinocardii* Pixell-Goodrich
Spatangus sp.	*Urospora echinocardii* Pixell-Goodrich
Synapta purpureus	*Lithocystis microspora* Pixell-Goodrich
Synapta galliennei	*Urospora synaptae* Cuénot
Synapta digitata	*Gonospora mercieri* Cuénot
Mollusca	
Cerithium vulgatum	*Gonospora testiculi* Trégouboff
Crustacea	
Ampelisca spinipes	*Cephaloidophora ampelisca* Kamm
Anaspides tasmaniae	*Ganymedes anaspides* Huxley
Atyaephyra Desmaresti	*Uradiophora cuenoti* Mercier
Balanus amphitrite	*Pyxinoides balani* Trégouboff
Balanus eburneus	*Pyxinoides balani* Trégouboff
Balanus eburneus	Unnamed parasite, Buddington
Eriphia spinifrons	*Porospora légeri* deBeauchamp
Gammarus marinus	*Cephaloidophora maculata* Léger and Duboscq
Libinia dubia	*Cephaloidophora olivia* Kamm
Nephrops norvegicus	*Porospora nephropsis* Léger and Duboscq
Portunus depurator	*Porospora maraisi* Léger and Duboscq
Pisa gibosii	*Porospora pisae* Léger and Duboscq
Talitrus saltator	*Cephaloidophora talitri* Mercier
Talorchestia longicornis	*Cephaloidophora delphinia* Kamm
Uca pugnax	*Cephaloidophora nigrofusca* Kamm
Uca pugilator	
Chilopoda	
Scolopendra heros	*Amphorocephalus amphorellus* Ellis
Scolopendra subspinipes	*Nina indicia* Merton
Scolopendra sp.	*Echinomera magalhães il* Kamm
Scolopendra sp.	*Seticephalus elegans* Kamm
Scolopendra sp.	*Gregarina brasiliensis* Pinto
Scolopendrella sp.	Gregarine form, Wellmer

Host	Parasite
Diplopoda	
Callipus lactarius	*Stenophora lactaria* Watson
Euryurus erythropygus	*Stenophora diplocorpa* Watson
Fontaneria coarctata	*Stenophora caudata* Watson
Orthomorpha coarctata	*Stenophora elongata* Ellis
Orthomorpha gracilis	*Stenophora robusta* Ellis
Orthomorpha sp.	*Stenophora robusta* Ellis
Orthomorpha sp.	*Fonsecaia polymorpha* Pinto
Parajulus impressus	*Stenophora impressa* Watson
Parajulus venustus	*Stenophora robusta* Ellis
Parajulus sp.	*Stenophora cockerellae* Ellis
Rhinocricus pugio	*Stenophora cunhai* Pinto
Rhinocricus sp.	*Stenophora lutzi* Pinto
Rhinocricus sp.	*Stenophora cruzi* Pinto
Rhinocricus sp.	*Stenophora viannai* Pinto
Rhinocricus sp.	*Stenophora umbilicata* Pinto
Rhinocricus sp.	*Stenophora tenuicollis* Pinto
Thysanura	
Sminthurus fuscus	Gregarine form, Wellmer
Orthoptera	
Ceuthophilus latens	*Gregarina longiducta* Ellis
Ceuthophilus maculatus	*Gregarina longiducta* Ellis
Ceuthophilus neglectus	*Gregarina neglecta* Watson
Ceuthophilus stygius	*Gregarina stygia* Watson
Ceuthophilus valgus	*Gregarina consobrina* Ellis
Conocephalus frater	*Gregarina chagasi* Pinto
Encoptolophus sordidus	*Gregarina nigra* Watson
Forficularia auricularia	Gregarine form, Pantel
Gryllus abbreviatus	*Gregarina galliveri* Watson
Ischnoptera pennsylvanicus	*Gregarina illinensis* Watson
Melanoplus differentialis	*Gregarina nigra* Watson
Melanoplus femur-rubrum	*Gregarina nigra* Watson
Udeopsyllae nigra	*Gregarina udeopsyllae* Watson
Hemiptera	
Spiniger sp.	*Schizocystis spiniger* Machado
Neuroptera	
Aeschnidae lv.	*Bothriopsis claviformis* Pinto
Aeschna sp.	*Actinocephalus brachydactylus* Ellis
Phryganea grandis	*Diplocystis phryganeae* Berg-von-Emme
Sympetrum rubicundulum	*Prismatospora evansi* Ellis
Tramea lacerata	*Prismatospora evansi* Ellis
Diptera	
Anopheles bifurcatus lv.	*Caulleryella anophelis* Hesse
Aphiochaeta rufipes lv.	*Caulleryella aphiochaetae* Keilin
Ceratophyllus fasciatus	*Agrippina bona* Strickland
Ceratophyllus farreni	*Steinina rotundata* Ashworth and Rettie
Ceratophyllus fringillae lv.	*Actinocephalus parvus* Wellmer
Ceratophyllus gallinae lv.	*Actinocephalus parvus* Wellmer
Ceratophyllus gallinae ad.	*Steinina rotundata* Ashworth and Rettie
Ceratophyllus styx	*Steinina rotundata* Ashworth and Rettie

Host	Parasite
Ficalbia dofleini lv.	Unnamed par. Guenther
Simulium bracteatum lv.	Unnamed par. Strickland
Stegomyia fasciaca lv.	*Lankesteria culicis* Stevenson and Wenyon
COLEOPTERA	
Alobates pennsylvanicus	*Actinocephalus zophus* Ellis
Amara angustata	*Steinina rotunda* Watson
Asida opaca	*Stylocephalus giganteus* Ellis
Asida sp.	*Stylocephalus giganteus* Ellis
Broscus cephalotes	*Gregarina erecta* Wellmer
Carabus sp.	*Cometoides* sp. Wellmer
Clerid lv.	*Bulbocephalus wardi* Watson
Coccinella sp.	*Gregarina fragilis* Watson
Coccinella sp.	*Gregarina katherina* Watson
Coccinella novemnotata	*Gregarina katherina* Watson
Coptotomus interrogatus	*Gregarina globosa* Watson
Coptotomus interrogatus	*Gregarina coptotomi* Watson
Crypticus quisquilius	*Gregarina ovoidea* Wellmer
Cucujus lv.	*Bulbocephalus elongatus* Watson
Cychrus rostratus	Gregarine form, Wellmer
Dermestes lardarius	*Pyxinia bulbifera* Watson
Diabrotica vittata	*Gregarina diabrotica* Kamm
Elateridae lv.	*Gregarina gracilis* Watson
Eleodes sp.	*Stylocephalus giganteus* Ellis
Eusattus sp.	*Stylocephalus giganteus* Ellis
Harpalus aeneus	*Gregarina polyaulia* Wellmer
Harpalus pennsylvanicus	*Actinocephalus gimbeli* Watson
Harpalus pennsylvanicus erythropus	*Hirmocustis harpali* Watson
Harpalus pennsylvanicus longior	*Steinina harpali* Watson
Harpalus ruficornis	*Gregarina polyaulia* Wellmer
Heledona agricola	Gregarine form, Wellmer
Helophorus aquaticus	*Monocystis* sp. Wellmer
Hydrophilus aterrimus lv.	Cometoides-like form, Wellmer
Hydrophilus sp.	*Bothriopsis terpsichorella* Ellis
Hylobius abictis	*Gregarina hylobii* Kamm
Ips typographus	*Gregarina typographi* Fuchs
Lagria hirta	*Gregarina rostrata* Wellmer
Leptochirus edax	*Actinocephalus crassus* Ellis
Leptochirus edax	*Stylocystis ensiferus* Ellis
Ninus interstitialis	*Gregarina guatemalensis* Ellis
Nyctotheres barbarata	*Actinocephalus zophus* Ellis
Omoplata normalis	*Gregarina watsoni* Pinto
Platydema excavatum	*Gregarina platydema* Kamm
Platynusruficollis	*Gregarina platyni* Watson
Procrustes coriaceus	*Actinocephalus permagnus* Wellmer
Pterostichus niger	*Gregarina exiguus* Kamm
Pterostichus niger	*Actinocephalus echinatus* Wellmer
Systena sp.	*Gregarina aragaoi* Pinto
Pterostichus stygicus	*Gregarina monarchia* Watson
Pterostichus stygicus	*Gregarina intestinalis* Watson
Pterostichus vulgaris	*Actinocephalus echinatus* Wellmer

Host	Parasite
Tenebrio castaneus	*Gregarina grisea* Ellis
Tenebrionidae lv.	*Gregarina tenebrionella* Watson
Tribolium ferrugineum	*Gregarina minuta* Ishii
Tribolium ferrugineum	*Gregarina crassa* Watson
Tribolium ferrugineum	*Disymophyes minuta* Kamm
Tribolium ferrugineum	*Steinina obconica* Ishii
Tritoma quadripustulata	Gregarine form, Wellmer
Lepidoptera	
Endrosis fenestrella lv.	*Leidyana tinei* Keilin
Oecophora pseudospretella Stain	Unnamed greg.
Tinea pallescentella Stain	Unnamed greg.
Arachnida	
Ctenocephalus serraticeps	*Gregarina ctenocaphalus* Ross
Oribata geniculata	*Gregarina* sp. Wellmer
Tunicata	
Stolonica socialis	*Selysina perforans* Duboscq
Enteropneusta	
Glossobalanus minutus	*Selenidium metchnikovi* Léger and Duboscq

DEPARTMENT OF METHODS, REVIEWS, ABSTRACTS, AND BRIEFER ARTICLES

ABNORMAL EARTHWORM SPECIMENS, *HELODRILUS SUBRUBICUNDUS* AND *H. TENUIS**

By
Frank Smith
University of Illinois

Comparatively little attention has thus far been given to abnormalities in the relations of the reproductive organs of earthworms. Variations from the normal positions and number are sometimes found, and asymmetrically placed gonads and openings of efferent ducts are not infrequently encountered. Since further investigation of such abnormalities may lead to at least a partial understanding of their relation to disturbances in the normal developmental activities of the animals concerned, it seems advisable to record the more important details in the structure of specimens representative of some of the more common types of such abnormalities, if indeed it be found that there are such types.

A specimen of *Helodrilus subrubicundus* (Eisen) recently collected at Urbana, Illinois, in the banks of a stream heavily contaminated with sewage was found to have the spermiducal pores on somite 14 instead of in the usual position on the fifteenth somite. Sagittal sections of the left half of the anterior part were made and unexpected irregularities were found. Spermaries and spermiducal funnels are present in the usual positions in 10 and 11. An ovary and oviducal funnel are present in the usual positions in 13; but an additional one of each, equally well developed, have similar positions in the twelfth somite which normally has no gonads. An oviducal pore is present in the usual position on 14, and in addition there is a supernumerary one on 13, related to the oviducal funnel of 12. The spermiducal pores on 14 are slightly laterad of the oviducal pores of the same somite. Sperm sacs in 9, 11, 12, and an ovisac in 14 have the usual location and relations. The calciferous gland, crop, and gizzard also have the usual location and relations; but the most posterior heart is in 10 instead of in the usual position in 11; and the lateral longitudinal vessel branches off from the dorsal vessel in 11 instead of in the usual place in 12. The ventral setae of 9 are modified to genital setae which are of about twice the length of ordinary setae and relatively more slender.

*Contribution from the Zoological Laboratory of the University of Illinois, No. 205.

The presence of extra gonads in 12 is not infrequently met with, but the presence of spermiducal and oviducal pores on the same somite (14) is decidedly unusual, in the experience of the writer, but has been found also in another specimen, described below.

A specimen of *Helodrilus tenuis* (Eisen) collected near Urbana, Illinois in a fallen and decaying tree, attracted attention because the spermiducal pores were asymmetrically placed, the one on the right side being normally situated on 15, while that of the other side opened on the somite next anterior. Sections were made and the asymmetrical relations were found to extend to internal organs. Reproductive organs of the right side were found in normal positions and relations, as follows: spermaries and spermiducal funnels in 10 and 11; an ovary and oviducal funnel in 13; oviducal pore on 14; and the spermiducal pore on 15. In the left half of the worm, there are spermaries and spermiducal funnels in 9, 10, 11; ovaries and oviducal funnels in 12 and 13; oviducal pores on 13 and 14; and a spermiducal pore on 14, laterad of the oviducal pore of that somite. The extra gonads and associated funnels are as large and well developed as the normal ones. Paired sperm sacs in 11 and 12 are in the locations normal for this species. No irregularities in the location of hearts and lateral longitudinal vessels have been noticed; and the alimentary tract has normal relations, except that the anterior evagination of the calciferous gland in the left half of the worm is found anterior to the septum 9/10, and the one in the right half is anterior to 10/11 which is the more normal position.

Asymmetry in the number and position of various organs in the right and left halves of specimens is of fairly frequent occurrence and often involves circulatory and alimentary systems as well as the reproductive organs. It will be noticed that the presence of both spermiducal and oviducal spores on 14 is associated, in the two specimens described above, with the presence of ovaries and oviducal funnels in both 12 and 13; but such association may be a mere coincidence rather than an actual correlation.

SUBSTITUTES FOR ABSOLUTE ETHYL ALCOHOL

By

Lawrence E. Griffin

Reed College

During the past year the writer has had his interest attracted to the question of whether other alcohols can be successfully substituted for anhydrous ("absolute") ethyl alcohol in histological work. For a considerable part of the year the air of Portland, Oregon, is nearly saturated with water vapor, and the tendency of absolute ethyl alcohol to absorb water constitutes one of the difficulties in its use. More potent reasons for seeking substitutes were, however, the high cost of the absolute ethyl alcohol and annoyances resulting from tax and prohibition laws. Even when alcohol can be secured by institutions free of tax there are regulations to be observed which entail a certain amount of delay in securing it, as well as much supervision of its use. So it would be advantageous if other alcohols can be used which are not subject to the regulations of the Bureau of Internal Revenue, and which are not sought as beverages. Fortunately, there are at least three such alcohols which have proved to possess merit.

METHYL ALCOHOL. The ordinary commercial quality of methyl alcohol contains about 95% of alcohol, the remainder consisting mostly of acetone, with traces of a large variety of other impurities. Purified methyl alcohol, anhydrous, and nearly free from acetone and other impurities, is put on the market under various trade names. The brand which we have tested is known as Diamond Methyl alcohol. Being practically free from acetone it not only lacks the strong disagreeable smell of ordinary wood alcohol but, in fact, has a pleasant odor much like that of refined grain alcohol. This alcohol dehydrates sections and tissues as well as the absolute ethyl alcohol, and is a little more reliable because it will dehydrate more sections than an equal amount of absolute grain alcohol. We have found it to be a good solvent, and have used it with success in the compounding of a number of reagents. As regards cost, it was not only far cheaper than anhydrous ethyl alcohol, but was considerably cheaper than the tax-free 95% grain alcohol which we bought a short time before we secured the methyl alcohol. We are now using this alcohol as our standard reagent in dehydration. In the course of eight months our stock, kept in a large glass stoppered bottle, has not absorbed enough water vapor to be noticeable. We purposely made no particular effort to seal the stock bottles tightly from the atmosphere. Anhydrous ethyl alcohol kept under the same conditions would have been useless for the dehydration of tissues.

BUTYL ALCOHOL. Professor George W. Martin has called attention (Science, April 21, 1922), to the use of butyl alcohol in dehydration and infiltration with parafin. This alcohol has been under test in our laboratory as a dehydrating reagent for several months and has given excellent results. We have used it, however, only in the last stage of dehydration, passing slides from 90% methyl or 95% ethyl alcohols to the butyl alcohol. It appears to us to be superior to either ethyl or methyl alcohols for dehydration, but its use is slightly disagreeable on account of the pungent, characteristic odor. Inhalation of its fumes causes a slight, temporary irritation of the throat. In reply to our inquiry as to whether this property of butyl alcohol might be removed, the Commerical Solvents Corporation, which made our sample, replied:

"The irritation of the throat, caused by the use of Butanol, is quite characteristic of this compound and is a property which it would be difficult to obliterate. However, we do not believe it has any harmful effect, as some of the men who work on the distillation end of the process have been subjected to this for years and have experienced no ill effects. They are much less susceptible to the irritation after having worked with this compound for some time."

Butyl alcohol is, at any rate, a valuable reagent for dehydration, our observation being that the sections dehydrated with it are slightly more brilliant than those cleared with the previously mentioned alcohols. As Professor Martin also states, butyl alcohol is a solvent of paraffin, and can be used for infiltration of tissues likely to shrink or harden in the usual infiltrants. We believe, however, that for this purpose it is surpassed by Terpeneol.

TERPENEOL. The use of terpeneol (terpineol) in place of absolute ethyl alcohol was suggested a number of years ago, but it is only lately that I have been able to test it thoroughly. Terpeneol is a pleasant smelling, aromatic liquid, of about the consistency of thin cedar oil. It is tolerant of large amounts of water in dehydration, and also dissolves paraffin and resins. On account of its consistency we have found it advisable to use first a mixture of terpeneol and methyl alcohol before placing tissues into pure terpeneol. Terpeneol may be used as a dehydrating agent for sections, but does not have any advantages over methyl or butyl alcohol when used in that way. As it dissolves parafin readily it is more useful as a dehydrant and infiltrant of tissues to be embedded. Terpeneol dissolves parafin better than butyl alcohol. Our experience has been that tissues which had been dehydrated and infiltrated with terpeneol were less shrunk and hardened than when embedded by the ordinary methods. Terpeneol is of rather high refractive index, so that it serves as a clearing agent also. Sections may be transferred directly from terpeneol to Xylol-damar. As the terpeneol

does not make tissues so brittle as does Xylol it can be used advantageously in the preparation of whole mounts.

We have also found that damar dissolved in terpeneol makes a mounting medium which, on account of the refractive index being lower than that of xylol-damar or xylol-balsam, shows some details of cell structure which are obscured in these commonly used mounting media.

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TRANSACTIONS
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Vol. XLI OCTOBER, 1922 No. 4

THE ANATOMY OF SOME SEXUALLY MATURE SPECIMENS OF DERO LIMOSA LEIDY.[1]

By
Roy L. Mayhew
University of Illinois

Introduction

Ecology. The material on which this paper is based was collected during the summer of 1921 at the Biological Station of the University of Michigan, on Douglas Lake, Michigan. The particular locality was a small bog draining into Burt Lake, about four and one half miles from the Station. A thick mat of algae taken from a plank at the surface of the water proved to be especially rich in the following genera of Naididae: *Nais*, *Chaetogaster*, *Pristina*, and *Dero*. The families Lumbriculidae and Enchytraeidae were represented by a number of specimens. Plankton taken from the water near by contained specimens of the genera *Slavina*, *Stylaria*, *Nais*, *Dero*, and *Pristina*.

During the examination of the algae it was frequently noticed that a number of worms of the same species would be found close together. Notes made at the time showed that four sexually mature specimens of *Dero limosa* were thus found in close proximity. At other times *Pristina* would be represented in great abundance. Five specimens belonging to the genus *Nais* were taken from an area about two inches square and were the only specimens of the genus found in the algae. Only one or two specimens belonging to the genus *Nais* were taken from the plankton. So commonly was the grouping of worms of the same kind observed, that when one of a particularly desirable type was located, the algae of the immediate vicinity was examined with special care.

Technique. Specimens of *Dero* were found to be the most difficult of any of the worms encountered, to kill and fix for histological study. Anesthesis with chloretone often resulted in the death and maceration of

[1] Contribution from the Zoological Laboratory of the University of Illinois, No. 212 and from the University of Michigan Biological Station.

the posterior part of the worm before the anterior ceased to move or came sufficiently under the influence of the drug to prevent bending and distortion when subjected to the killing fluid. A concentrated aqueous solution of corrosive sublimate was used as a fixing fluid. The specimens sectioned were cut transversely, 6μ in thickness, and stained with Delafield's haematoxlin and eosin. Mounts, in toto, were stained in borax carmine and cleared in oil of wintergreen.

Identification. Seven sexually mature and numerous immature specimens of *Dero limosa* Leidy were taken from the algae referred to above during the first two weeks of August, 1921. The determination of the species proved a little difficult since living specimens not anesthetized were constantly in motion, and, when anesthetized or killed for preservation, were usually found to have completely closed the branchial pavilion. The gill arrangement seems, however, identical with that of the above named species as described by Michaelsen ('00, '03, & '09) and others. Examination of sections of the closed pavilion reveals the fact that the lateral portions of the lip, which are carried medially in the process of closing, may easily be mistaken for a third pair of gills on the ventral portion of the structure, since they are intimately associated with the true gills in position and have similar cell arrangement (fig. 16).

Since the only descriptions found of sexually mature specimens belonging to the genus are those given by Michaelsen ('00) for *D. perrieri* and *D. multibranchiata*, and by Walton ('06) in his general description of the genus, it seems desirable to give a rather extended account of the new material mentioned above. The descriptions of the sex organs as given by Michaelsen are brief and are here quoted for comparison.

D. perrieri, "1 Paar Samentrichter im 5. Segm.; Samenleiter allmählich in die drüsenlosen Atrien übergehend. 1 unpaariger Eiersack. Samentaschen im 5. Segm., mit scharf abgesetztem, ziemlich kurzem, tonnenförmigem Ausführungsgang."

D. multibranchiata, "Unpaariger Samensack vom 5. Segm. nach hinten gehend. Ovarien (Eiersäcke?) im 7. Segm."

Length and Number of Somites

The length of preserved sexually mature specimens varied from 5 to 10 mm. and the total number of segments from 40 to 55. Immature specimens showed an equal range of variation in length and contained from 40 to 62 somites; those with one or two budding zones containing from 50 to 59.

Setae

In respect to the setae, the sexually mature specimens differ from the immature only in the entire absence of ventral bundles in the 6th somite

in contrast with the occurence of special or genital setae in *Nais obtusa* and a number of other species described by Piguet ('06 & '09). The dorsal bundles, which begin on the 6th somite in both mature and immature specimens, have been found to contain one long capilliform and one short, slightly cleft, biuncinate seta in each of the bundles examined (fig. 13). The long capilliform setae are about equal in length to the diameter of the body, and are without serrations. The biuncinate setae extend but little beyond the surface of the body, and are more or less curved and somewhat tapered distally. Their distal portions are much stouter than figured by Michaelsen ('09) and by Bousfield ('87), and the tips of the teeth are not sharp pointed but slightly rounded. One biuncinate seta of a sexually mature specimen was found to be somewhat smaller in diameter and to have a slight nodulus. Piguet ('09) says that mature specimens of the genus *Nais* lose the setae of the dorsal bundles in the clitellar somites, but such is not the case in *D. limosa*.

The ventral bundles (fig. 9–12) contain from 2 to 5, in the majority of instances 3 or 4, biuncinate setae. The distal tooth, or the one farthest from the nodulus, is about equal in length to the proximal, or but slightly longer. The teeth are usually sharp pointed, though occasionally the proximal one is thickened and round pointed. They often appear to have round tips due to their being turned so that an exact lateral view is not obtained. Each of the ventral setae is provided with a well defined nodulus.

The ventral setae of somites 2–5 are longer than those from the remainder of the body, in both mature and immature specimens. Setae from each of the somites 2–5 were measured and averaged for each specimen with the result that the averages varied from 120μ to 160μ. Averages obtained in like manner from a number of somites posterior to 6 varied from 80 to 100μ. The extreme difference between the averages so obtained for any specimen was 65μ, the measurements being 95μ to 160μ. Definite ratio relations have been noted between the portions of the setae proximal and distal to the noduli in particular parts of the worm. The portions of the setae distal to the noduli in somites 2–5 are usually about equal in length to the proximal parts but may be as much as 1.5 times as long, while the distal portions of setae in somites posterior to 6 are only .6 to .8 as long as the proximal.

The Reproductive Organs

Clitellum. This organ extends from the anterior part of the 5th somite almost to the setae of the 8th (fig. 1, cl.). On the ventral side it begins just back of the openings of the spermathecae, but dorsally farther forward. In life the clitellum is yellowish or cream colored, and, when sectioned, is found to be made up of a single layer of large columnar cells

containing large globules of a substance, which is probably the secretion that later forms the cocoon, and relatively small nuclei irregularly distributed in the cells.

Spermathecae. These are conspicuous paired organs almost filling the ventral two thirds of the 5th somite (fig. 1–3, spm.) The upper part is a relatively thick walled sac with deeply staining, irregularly arranged nuclei, and well filled with a compact mass of sperm cells indicating that copulation has taken place. The duct arises a little laterad of the most ventral portion of the sac and extends directly to the body wall where it turns directly posteriad, a very short distance, and opens on the surface through the pore slightly anteriad and laterad of the ventral seta bundle of the same side of the somite (fig. 2 & 3). In one specimen the duct passes almost directly to the pore. The walls of the duct are thickly set with nuclei of about the same size as those of the sac. Dorsal to the spermathecae there is a quantity of sperm cells in the body cavity.

Sperm ducts. A pair of sperm ducts lie in the 6th somite and have their funnels in the posterior part of the 5th. Each of the pair consists of four portions, (1) the spermiducal funnel, (2) a duct joining the funnel to the atrium, (3) the atrium, and (4) the duct connecting the atrium with the hypodermal invagination. The spermiducal funnels are found in the ventral posterior part of the 5th somite anterior to septum 5/6. They are shaped as if the distal end of the duct had been split on the dorsal side and the lateral portions flattened out in the process of formation. The part of the duct between the funnel and the atrium tapers posteriorly, bends abruptly upon itself (figs. 1 & 7), and extends anteriorly to its point of union with the atrium, on the median anterior ventral surface of the latter. The atrium is a cylindrical sac occupying much of the anterior ventral portion of the corresponding half of the 6th somite. The walls are relatively thick and are made up of a single layer of large irregular cells containing large vacuoles. The duct leading from its posterior end is short and opens into a conspicuous invagination of the hypodermis on the ventral wall of the body. In one specimen one atrium was displaced so that it lay dorsad of the nerve cord in the median line of the body.

Spermaries. Nothing was found which could be identified as such. This fact is probably due to the advanced stage of sexual maturity of the specimens, as the sperm sac and spermathecae were filled with sperm cells. When developed, they should be present on the posterior side of septum 4/5, since sperm cells were found above the spermathecae, and the sperm sac extended posteriad from the 5th somite.

Sperm sac. The sperm sac is a posterior evagination of septum 5/6, and extends a short distance posteriad of the setae of the 7th somite in one specimen.

Oviducal pores and funnels. The pores are paired and are in the posterior part of the 6th somite about midway between a line joining the ventral seta bundles and one joining the dorsal seta bundles of the 6th and 7th somites (figs. 1 & 14). No distinct funnels could be identified comparable with those figured and described by Piguet ('09) for *Nais obtusa*, although there appeared to be several cells on each side which might properly be interpreted as belonging to a funnel since they are sharply differentiated from the clitellum (fig. 15). The cells of the clitellum are so graded in length as to form a distinct depression with the funnel cells at the base. The paired funnels are in the same relative position as those of *Nais obtusa*. The lumen of the pore could not be located, probably because of the thickness of the transverse sections, and, for the same reason, the opening in the muscular layers was not observed. The muscular layers were found separated from the pore cells in that region. The funnel and pore would no doubt be much more conspicuous at the time of emission of the eggs.

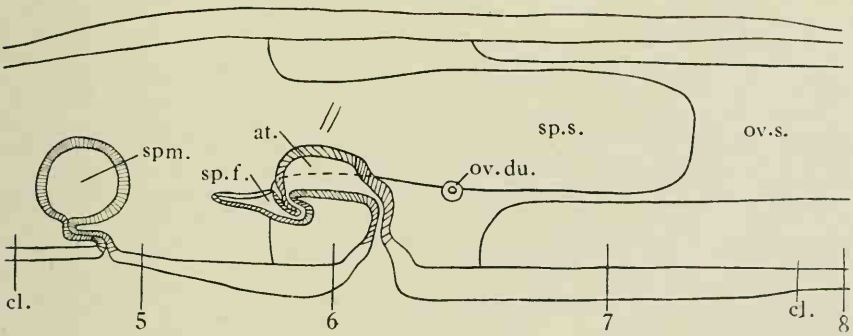

FIG. 1. Diagram showing the general plan of arrangement of the sex organs in somites 5 to 8. Reconstructed from camera lucida outlines of serial transverse sections. Lettering as for other figures. The numbers indicate the position of the setae bundles. 120X.

Ovisac. (fig. 1). The ovisac is formed by septum 6/7 and extends posteriad, in one specimen through the 12th somite, in another, just beyond the seta bundles of the 9th. Its extent is no doubt dependent upon the quantity of ova present. It almost fills the body cavity for the major part of its length, and is distended with a granular appearing material which stains pink with eosin, but contains no ova. The sperm sac lies within its anterior portion.

Ovaries. No ovaries could be found. They should be located on the posterior side of septum 5/6 since the ovisac extends posteriad from 6 and the oviducal pore is in the posterior part of this somite (fig. 1). Their absence is no doubt due to the advanced stage of development of the specimens. The absence of ova and the presence of abundant sperm cells suggests the possibility that an interval of time elapses between the

functioning of the spermaries and ovaries. However this does not seem probable because of the very extensive development of the ovisac. It seems more probable that ova have occupied the latter and have been discharged.

Piguet ('06) refers to the appearance of gonads as follows: "Michaelsen suppose que, chez les Naïdidées, les gonades disparaissent entièrement avant le développement des autres organes génitaux; cela est sans doute vrai en général, mais il pourrait y avoir là une exception. Frank Smith (1896, Pl. 35, fig. 4, t.) figure un reste de testicule chez *Pristina Leidyi*." In several mature specimens of *Paranais uncinata* Piguet has observed vestiges of ovaries. It seems, therefore, that there are individual exceptions, but the few observations that have been possible upon *Dero limosa* indicate that gonads are developed only during the period of production of the germ cells.

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ABBREVIATIONS

at.	Atrium
at. 1	Posterior end of atrium
b. l.	Borders of dorsal lip
cl.	Clitellum, in fig. 1 the extent of the organ is indicated
du. s.	Duct of spermatheca
gil. 1	True gills
gil. 2	Secondary gills
hyp.	Hypodermis
int.	Intestine
mus.	Muscular layers
nc.	Nerve cord
ov. du.	Oviducal funnel and pore
ov. s.	Ovisac
sp.	Sperm cells
spm.	Spermathecae
sp. du.	Sperm duct
sp. du. 1	Sperm duct opening into atrium
sp. du. 2	Sperm duct in transverse section at its bend
sp. f.	Spermiducal funnel
sp. s.	Sperm sac

DESCRIPTION OF FIGURES

Fig. 2. Transverse section in the 5th somite showing spermathecae. 120X.

Fig. 3. Portion of left spermatheca, its duct and adjacent body wall in transverse section. 270X.

Fig. 4. Transverse section of spermiducal funnel with sperm cells. 270X.

Fig. 5. Transverse section of spermiducal funnel posteriad of section represented in fig. 4. 270X.

FIG. 6. Transverse section of sperm duct and anterior end of atrium at the point of entrance of the duct. 270X.

FIG. 7. Transverse section of the left ventral portion of a specimen at the point where the sperm duct bends anteriad. 270X.

FIG. 8. Transverse section of the left ventral portion of the same specimen, as represented in the preceding figures, at the point where the hypodermal invagination receives the sperm duct. 270X.

FIG. 9. Left ventral setae bundle of the 7th somite. 270X.

FIG. 10. Left ventral setae bundle of the 30th somite. 270X.

FIG. 11. Left ventral seta of the 2nd somite. 270X.

FIG. 12. Left ventral seta of the 3rd somite. 270X.

FIG. 13. Right dorsal setae bundle on the posterior half of a specimen. 340X.

FIG. 14. Diagram showing the position of the oviducal funnels. The position of the setae of the 7th somite is shown, as obtained by superimposing the sections containing them (21 sections posteriad) upon the outline of the sections containing the funnels, by means of a camera lucida. 120X.

FIG. 15. Diagram showing the differentiation of the oviducal funnel from the clitellum 270X.

FIG. 16. Transverse section of the closed branchial pavilion. 150X.

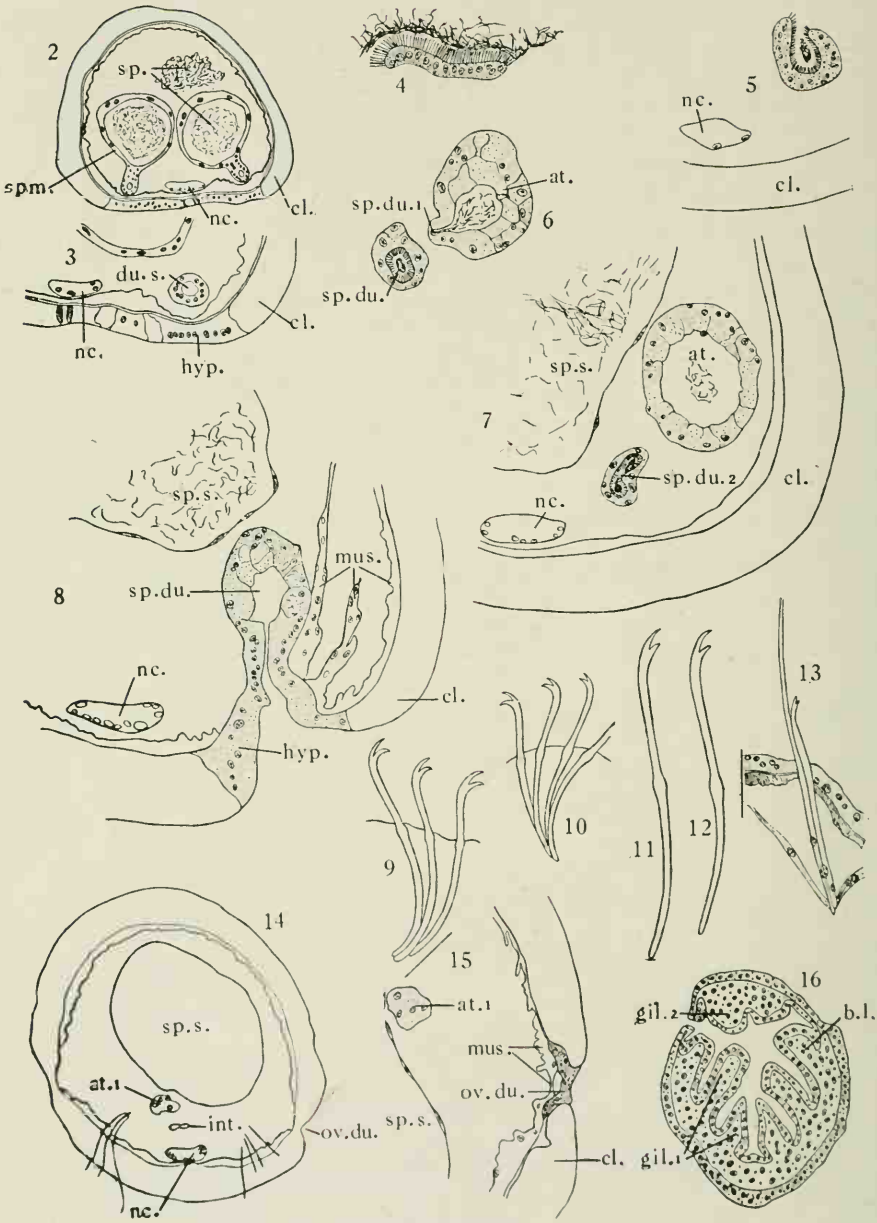
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PLATE XVI

STUDIES ON AMERICAN NAID OLIGOCHAETES

1. Preliminary Note on Naids of Douglas Lake, Michigan[1]

By
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University of Richmond

During July and August, 1921, I was engaged in a study of the Naididae of the region around the University of Michigan Biological Station on Douglas Lake in the upper end of the southern peninsula. This locality afforded a wealth of material for such a study, including a number of sexually mature forms. A full discussion of the systematic aspects of this work is in course of preparation, to be followed by papers dealing with the morphology and histology of the various naids, especially of the mature individuals.

As a preliminary report on this work, I wish to place on record here, for the benefit of other students of this family of the Oligochaeta, a list of the species noted, together with brief diagnoses of two new species and notes on two new varieties. The following established species were represented by forms which did not differ appreciably from the published descriptions:

Aulophorus furcatus (Oken)
Chaetogaster diaphanus (Gruithuisen)
Chaetogaster langi Bretscher
Chaetogaster limnaei K. von Baer
Dero limosa Leidy
Dero perrieri Bousfield
Nais communis Piguet
Nais pseudoöbtusa Piguet
Nais simplex Piguet
Nais variabilis Piguet
Pristina longiseta Ehrenberg
Slavina appendiculata (d'Udekem)
Stylaria fossularis Leidy
Stylaria lacustris (L.)
Vejdovskyella comata (Vejdovsky)

[1]Contribution from the University of Michigan Biological Station, and contributions from the Biological Laboratory of the University of Richmond, No. 1.

With the exception of *Stylaria fossularis* and *Vejdovskyella comata*, all these species were found in considerable numbers. Of these two species only one individual of each was found. In each case, however, the species is unique and its characteristics sufficiently pronounced to prevent any error in the identification.

The following species was represented by forms which showed only one marked difference from the type.

Pristina aequiseta Bourne

The individuals of this species agreed closely with the description of *Naidium tentaculatum* given by Piguet (1906), which species was later united by the same writer (1909) with *Pristina aequiseta* Bourne (1891). In the forms described by Piguet and Bourne, however, there are peculiarly enlarged setae in the ventral bundles of segment 4; while in the forms that came under my observation these setae were, with one exception, on segment 5. Whether this amounts to a varietal difference or whether the position of these setae is a matter of no importance, remains to be seen.

The following is, in my opinion, entitled to rank as a variety:

Chaetogaster diaphanus, var. *cyclops*, var. nov.

This is in most respects similar to the type form of the species, but differs from it in the presence of a very definite median pigmented body intimately associated with the brain and strikingly like an eyespot.

The following species have not hitherto been described:

Dero polycardia sp. nov.

Worms quite large, 7–10 mm. in length, about 300 microns in diameter. Color reddish. Swimming actively. Ventral setae of segments 2–5, four to six in number, about 135 microns long, nodulus proximal, distal tooth longer than proximal and with a slight swelling at base. Ventral setae of other segments, four to six in number, about 95 microns long, nodulus a trifle distal, teeth about equal, distal tooth half as thick as proximal, and with a slight swelling at base. Dorsal setae beginning on segment 6, with one or two capilliform setae, somewhat longer than the diameter of the body, and one or two needle-like setae, about 87.5 microns long, slender, bifid, nodulus distal, distal tooth longer than proximal, proximal part of the seta almost straight, distal part strongly curved. Contractile transverse vessels ("hearts") up to eight pair, in segments 6–13 inclusive, though one or more of the last few pair may be lacking. Blood quite red. Intestinal dilation in segments 9 and 10. First nephridia in segment 7. Respiratory bursa with dorsal lip, consisting of a median portion and two lateral ciliated processes. Gills, two pair, of the pyramidal type. Budding takes place between segments 25 and 36. Sexually mature forms

not yet observed. Habitat, in felted masses of blue-green algae attached to slightly submerged logs in a marshy pond near Burt Lake, Michigan.

Haemonais ciliata sp. nov.

Worms large, as much as 16 mm. in the case of double chains, but able to contract to about one third of their length. Diameter, about 500 microns. Very active and, because of their rapid contractions and expansions, rather leech-like in their movements. Color, light reddish. Number of segments up to 55 in individual worms, and up to 100 in double chains. Prostomium rather acuminate; when expanded, slightly longer than broad at the base. Eyes absent. Prostomium covered with fine, straight tactile processes; a zone of similar processes around each segment. Remainder of body surface bears frequent smaller processes which are sharply reflexed and terminate in a bulbous swelling. As far back as the first segment bearing dorsal setae, body surface ciliated. Setae about middle of segment. Ventral setae usually three in number, about 90 microns long, sigmoid, nodulus about middle, teeth equal in length, distal tooth half as thick as proximal, and with a slight swelling at base. In all the individuals observed, nine in number, the first four or five segments were very short, and the setae of these segments, while having the same form as those following, were relatively smaller. Dorsal setae beginning on any segment from 14 to 22 inclusive: with one capilliform seta, about 160 microns long, slightly sigmoid, distal half more curved than proximal; and one biuncinate seta, about 110 microns long, slightly sigmoid, nodulus barely distal, teeth long, distal tooth longer and a trifle thinner and with a very slight swelling at base. Pharynx short, pigmented at both ends. Remainder of canal not highly specialized. Contractile transverse vessels ("hearts") in most of segments 4–20 inclusive. Circulatory system more like the usual naid type than that of *H. waldvogeli* Bretscher (1900). Budding takes place after segment 40. Mature forms not yet observed. Habitat, in felted masses of blue-green algae attached to slightly submerged logs, and in water-macerated wood, from a marshy pool near Burt Lake, Michigan. In Bretscher's (1900) description of *H. waldvogeli*, no mention is made of the presence of cilia on the body surface, and as this is such a noticeable feature of *H. ciliata*, I have ventured to indicate this fact in the specific name.

The attention of systematic zoologists is called to the existence in this country of representatives of two genera not given in the key to the Naididae on pages 638–640 of Ward and Whipple's "Fresh-Water Biology": namely, *Haemonais* and *Vejdovskyella*. The latter will be found in Michaelsen (1909), as well as, under the older name *Bohemilla*, in Michaelsen (1900). *Haemonais*, hitherto known only through the single species, *H. waldvogeli*, is described in Bretscher (1900). These two genera may be

added to the key in Ward and Whipple by altering the text of page 639 as follows:

10 (11) Setae of dorsal bundles all uncinate ..... *Paranais* Czerniavsky 1880.

11 (10) Dorsal setae nearly straight, slightly toothed or simple-pointed..... *Ophidonais* Gervais 1838.

12 (9) Capilliform setae present in dorsal bundles.....13.

13 (13½, 21) First anterior dorsal setae on XII to XXII ..... *Haemonais* Bretscher 1900.

13½ (13, 21) First anterior dorsal setae on V or VI.....14.

14 (18) Posterior end not modified into a gill-bearing respiratory organ.....15.

15 (15½) Capilliform setae of dorsal bundle with a series of very prominent teeth; first anterior dorsal setae on V. ..... *Vejdovskyella* Michaelsen 1903

15½ (16, 17) Capilliform setae without teeth; one or more capilliform setae of VI much longer than those of other somites and equal to three or four times the diameter of the body..... *Slavina* Vejdovsky 1883.

16 (15½, 17) Prostomium elongated to form a proboscis; dorsal setae of VI similar in length to those of other somites ..... *Stylaria* Lamarck 1816.

17 (15½, 16) Without proboscis; dorsal setae of VI similar in length to those of other somites..... *Nais* Müller 1774.

18 (14) Posterior end modified into a gill-bearing respiratory organ, the branchial area.....19.

19 (20) Ventral margin of the branchial area with a pair of long processes..... *Aulophorus* Schmarda 1861.

Two ecological notes may be made very briefly here. The observation of Mrazek (1917) as to the ingestion of trematode larvae by *Chaetogaster limnaei* is similar in all respects to observations made in the course of my study of this form in Michigan. *Chaetogaster* is in general carnivorous, especially *Ch. diaphanus*. This latter species, particularly the *cyclops* variety, is actively predaceous and even cannibalistic, and those who are just beginning the study of these worms are warned to keep their chaetogasters away from vessels containing other genera, as they will depopulate a culture of naids in a very little time.

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EXCESSIVE SEXUAL DEVELOPMENT IN HYDRA OLIGACTIS WITH SPERMARY ON TENTACLE

By
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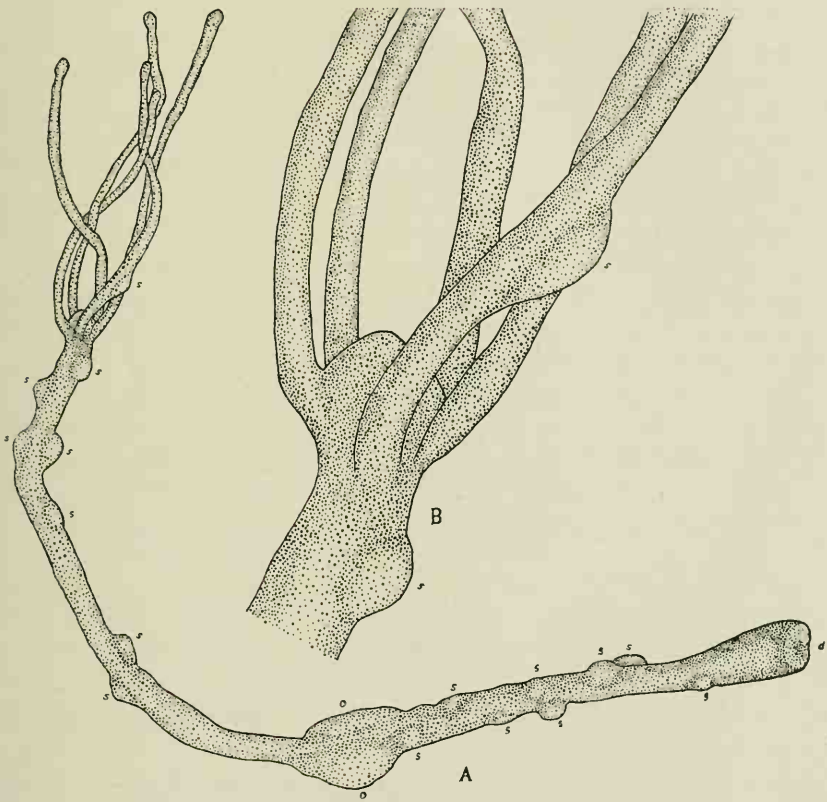
Department of Zoology, University of Nebraska[1]

This specimen of *Hydra oligactis* was found in an aquarium with other normal individuals of the same species in February of 1918. The culture was abundantly supplied with food, such as Daphnia, Cyclops, and various other Crustaceans. The specimen was killed with a solution of corrosive sublimate and acetic acid, stained in a special preparation of borax carmine, and mounted in Canada balsam. It measures 11 mm. in length, the body 8½ mm. and the tentacles 2½ mm. as it is mounted on the slide. Its extreme length when living was 13 mm. Fifteen spermaries and two ovaries occur on the body and one spermary on one of the tentacles of the specimen (See figure). The spermary on the tentacle appears to be perfectly normal in all respects except location. The question now arises as to the cause of its occurrence on the tentacle.

Parke cites the following instances in the establishment of normal tentacles from abnormal ones. "A nine-tentacled Hydra fusca with one branching tentacle was isolated on February 27th. On February 28th one branch had revolved about 45° till it was in line with the longitudinal axis of the tentacle, while the other one appeared somewhat shorter than it did at first. On March 1st the small branch was almost entirely resorbed. It was much nearer the end of the tentacle than before and appeared as a small outgrowth from the tentacle. This apparent shifting of the small branch, by a migration of the short branch, may have taken place in three ways: by a shortening of the long branch, by a migration of the short branch towards the end of the tentacle, or by a fusion of the two branches along the median line. The first or last explanation seems the most plausible since similar instances were seen in which there could be no doubt that these were the processes involved. On March 3rd the small branch was entirely resorbed, leaving the Hydra with nine normal tentacles. Two other instances of regulation of forked tentacles in the same manner were observed." He states further that his observations show that branching tentacles may arise by the fusion of two tentacles and may regulate themselves by a complete fusion along their median sides so as to form a single tentacle.

[1] Studies from the Zoological Laboratory, The University of Nebraska, No. 130.

In further observations he found that by regulative processes two distinct tentacles could be reformed out of two fused tentacles. He cites several instances in which two of the tentacles were fused near their ends and not near their bases along the median line. "This fusion," he states, "may have been caused by an injury to one of the tentacles, the other tentacle having become attached to it. This seems probable from the fact that alternate tentacles as well as adjacent tentacles were found fused in this manner, ... One eight-tentacled Hydra was found



Hydra oligac is: A, showing 18 gonads; *s*, spermary; *o*, ovary; *d*, basal disc. B, showing position of spermary on tentacle.

in which two alternate tentacles had stuck together at a point about three fourths of the distance from the base to the tips of the two tentacles at the point of fusion. There was no connection between the cavities of the two tentacles at the point of fusion. The next day after the Hydra had been isolated, one of the tentacles had constricted off from the other tentacle just below the point of fusion of the two, leaving the tip of its tentacle attached to the other branch. The cavities of the two branches

were in direct communication. The process of regulation that now took place was exactly as in the Hydra described above. This is a good example of how branching tentacles may originate." Both tentacles became normal. He summarizes his conclusions with the statement: "It appears that three regulative processes may take place in the establishment of normal tentacles; viz. (1) fusion, (2) resorption, (3) constriction."

The theories of fusion and constriction suggest the idea that by a contact of the tentacle with the spermary in its original position on the body of the Hydra the two were fused; and then by a process of constriction the spermary was severed from the body, leaving it in its present position on the tentacle. Parke's reference to the *migration* of a short branch of a tentacle, mentioned above, also suggests the idea that the spermary *migrated* from an original position on the body to its present position on the tentacle. Probably the most plausible suggestion, however, is that the position of the spermary is due to an unusual local stimulation of a group of interstitial cells in the ectoderm of the tentacle, causing abnormal development in that location. Due, however, to the absence of facts regarding previous conditions in the aquarium, it is impossible to throw any light upon the real cause of this abnormal condition, except by way of suppositions from previous investigations.

Whitney, in his observations on Hydra viridissima, found that when they are subjected to a low temperature and starvation they develop testes and eggs. Hertwig, however, found that if Hydra oligactis is kept at a temperature of 8°–10° C. it will develop testes irrespective of the food conditions. At the time of the discovery of this specimen of Hydra oligactis that was about the temperature of the room in which the aquarium that contained this specimen was kept.

Extensive experimental work on sexually reproducing Hydras would undoubtedly reveal the generality or abnormality of this occurrence.

The writer desires to express his thanks to Drs. Robt. H. Wolcott and David D. Whitney for access to materials and laboratory facilities, also for their suggestions and generous interest.

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SOME SUGGESTIONS FOR TEACHING MYCOLOGY

By
F. D. Heald

The study of any group of plants as to its taxonomy may proceed along two widely divergent lines: First, the student may be taught to use artificial keys and determine species, which are put in their respective species pigeon-holes and properly labeled, the prime object being to determine the binomial, but little concern being given to the relationship of the various forms studied; or Second, the student may be taught to construct diagrammatic keys to the various groups, which will express natural relationship. These natural keys, which give a graphic representation of relationship, are clearer than the obscurely worded artificial keys crowded full of technical terms. The writer has followed the latter method with marked success in presenting the taxonomy of seed plants with successive classes through a period of years and more recently has used the same plan with classes in mycology. The method has a number of features to recommend it, some of which are: (1) The creation of a greater interest on the part of the student in his work; (2) The development of the students' ability to reason and weigh evidences; (3) The cultivation of the scientific imagination; (4) A better understanding of evolution and what it means; and (5) The possibility of emphasizing natural descent of the various groups and bringing out the fact that classification is in reality but a means to an end,—an expression of relationships.

The work in mycology in our laboratory is offered to students who have had general elementary botany and also to those who have had in addition a semester in general pathology, in both of which they gain some familiarity with fungi. The minimum time which suffices for anything like a satisfactory presentation of the subject is six hours of laboratory work throughout the year. The general method of procedure may be briefly presented.

Very early in the beginning of the work the class is given a skeleton outline of the great groups, somewhat as shown in the accompanying diagram (Fig. 1), except that the diagrams are omitted and the student is required to select diagrams and make any adjustments that may seem necessary to present concepts of the great groups by the visual channel, or to bring out more clearly the natural relationships. Various mycological works, such as Engler & Prantl, Rabenhorst's Kryptogamen Flora, special monographs, etc. must be available for reference. Suggestions are

given to the student, but they are encouraged to use their own originality and independent thought as well as to consult authorities. They are also

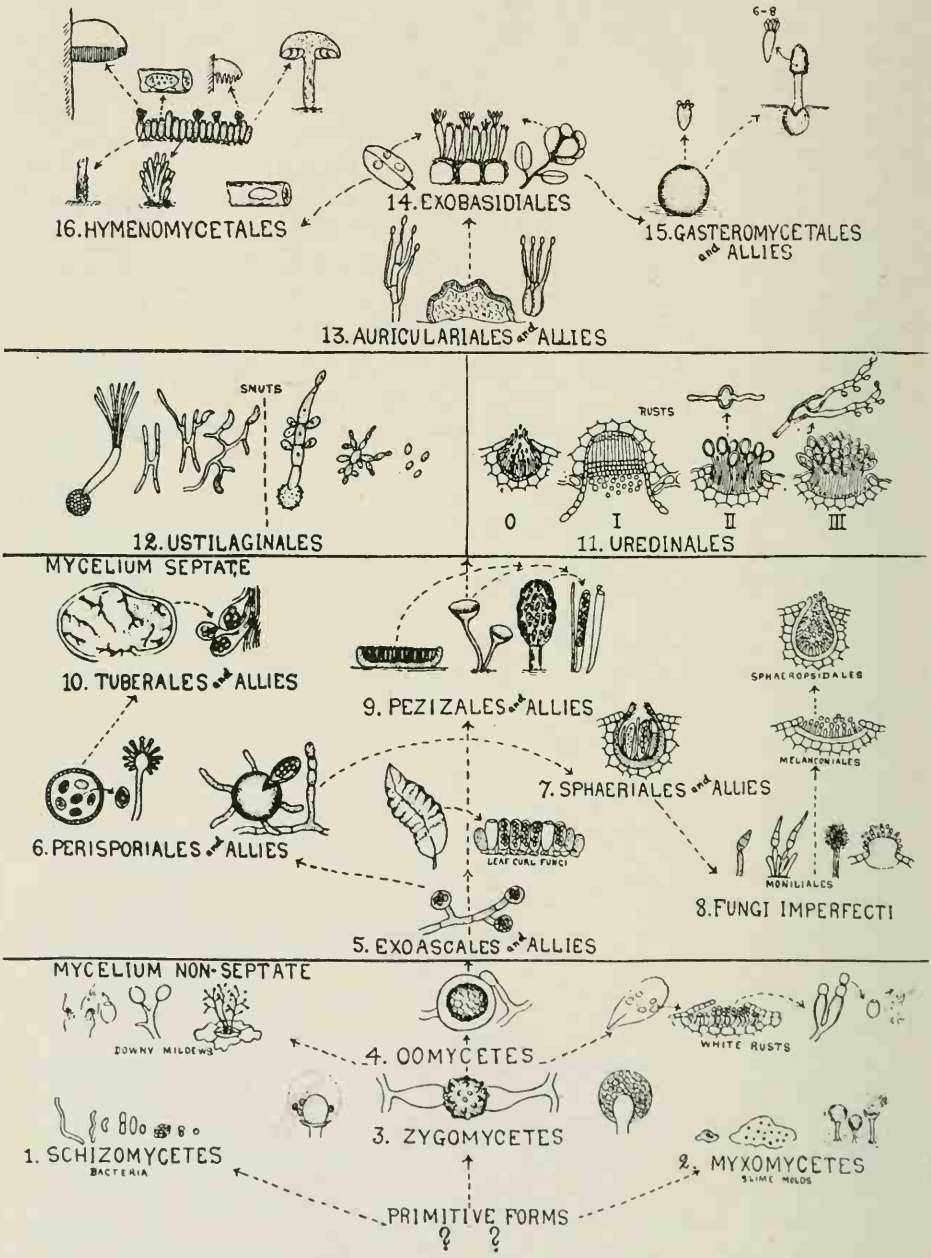

FIG. 1. Chart Showing the Great Group of Fungi.

given the understanding that schemes of natural relationship can be nothing more than the expression of individual opinion, which should be arrived at by weighing all the evidence that can be brought to bear in any specific case. No two students will choose the same illustrations and the more intimate details of relationship as expressed in the charts are certain to show variations. These variations afford excellent material for class discussions which can frequently be held with much profit.

The logical order for the study of the great groups would be to begin at the bottom of the family tree with the most primitive forms and proceed to the more complex and higher forms later. In actual practice, however, it seems better to sacrifice logic and begin with some group which more readily lends itself to the method in question. The Erysiphaceae, or powdery mildews of the order Perisporiales, is a family well suited to introduce the plan of study: (1) Because species determination is relatively easy; (2) Because representatives of all the genera can very readily be obtained in most environments. Our plan would call for a careful and detailed study of some type of each genus, accompanied by drawings. Following this the student is asked to construct a diagrammatic key to the genera of powdery mildews, which will express relationship and afford generic concepts, mainly through the visual channel. Before this key is made, a general class discussion is held and the more important characters which may indicate relationship are briefly reviewed, with emphasis on those which are primitive and those which are more advanced. These keys are then presented for comparison and discussion (Fig. 2). After the completion of the keys, the class is asked to determine the species of all the powdery mildews which they have collected on some of their special field trips.

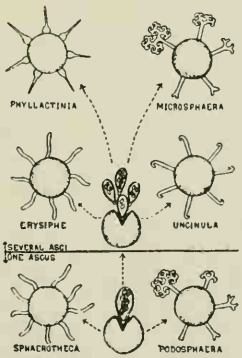

FIG. 2. Chart Showing the Genera of Powdery Mildews

Essentially the same plan is followed with all the great groups or alliances, or with representative families of these groups. It will be at once evident that all groups can not be treated as fully as the Erysipha-

ceae, which we have used for our introduction to the method. For example, in the study of the Sphaeropsidales of the Imperfect Fungi, attention is given to the genera furnishing parasites and only these are included in the graphic key which the students are required to construct. In other cases, as in the Sphaeriales and allies with numerous families, the graphic keys may be limited to a representation of the families.

As previously stated, the minimum time for a course in mycology according to the plan outlined is six hours of laboratory work per week throughout one school year. With this minimum time there must of necessity be many omissions and consequently much of the success of the course depends on the judgment of the instructor in making wise selections. There is no doubt that the same plan could be followed with much profit throughout an additional year of work.

This brief note has been prepared at the suggestion of several of my former students who have been stimulated to further mycological study by the use of the method outlined. It is hoped that it may offer some suggestions to some of our younger mycologists who have received their instruction by the pigeon-hole method.

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STUDIES ON *SPARGANOPHILUS EISENI* SMITH*

By Florence S. Hague

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I. Introduction

The genus Sparganophilus was established by Benham (1893) when he described the first species, *S. tamesis*. He found the specimens of this species in one restricted area along the Thames River. Since they had not been reported from other places in England, and since he was unable to

*Contributions from the Zoological Laboratory of the University of Illinois, No. 215.

find them in other places along the River, he concluded that they had been introduced there from some other part of the world. He thought it possible that they had been brought with timber or plants from North America, for America was considered the home of the most closely related forms. In 1895, H. F. Moore reported the presence of this same species in the banks of streams in the vicinity of Philadelphia. However, some of the Philadelphia worms were examined by Michaelsen (1917) and, although they were not adequately preserved for dissection, he found from external characters that they were not *S. tamesis*. In response to a request for material Doctor J. P. Moore, also of Philadelphia, wrote that he was unable to find any of the worms in the spring of 1920, in places where they had previously been found.

The next species reported was *S. eiseni*. The specimens were found in the Illinois River at Havana, Illinois, by Professor Frank Smith, and described by him in 1895. *S. eiseni* has since been reported from Ohio, Michigan, Indiana, and Florida. Eisen in 1896 described *S. benhami* from Mexico; *S. guatemalensis* and *S. carneus* from Guatemala and Iowa, respectively; *S. smithi* and *S. sonomae* both from California. The genera which, according to Michaelsen (1917), are most closely related to Sparganophilus are found in Central and South America.

This paper deals with certain organs and with variations in specimens of *S. eiseni* from Florida, Iowa, Illinois, and Michigan, from which states the genus has previously been reported, and also with specimens from Louisiana and Wisconsin. Although certain points in embryology and in the anatomy of several systems are given some attention, the subsequent discussion is concerned chiefly with the excretory and reproductive systems. The structure of a nephridium of *S. eiseni* is compared with that of the nephridia of other genera which have been studied. Although the development and subsequent loss of the nephridia of the anterior somites has been known for some time among limicoline Oligochaeta, a similar process has been reported in an earthworm only once. The detailed developmental history of the nephridia of the anterior somites of an earthworm is here presented for the first time. It has been found that they develop and then disintegrate while the worms are still quite young. The disintegration of the nephridia in the somites which contain the genital ducts throws additional light on the relation of genital ducts and nephridia. While accessory reproductive glands have been reported in numerous species of earthworms and their structure described in some of these species, the published accounts have been based on comparatively few specimens, and have not dealt with their development. The data in the present paper have been taken from over 150 specimens which were different in age and which were collected at various seasons of the year.

The writer is indebted to Doctor C. P. Alexander of the State Laboratory of Natural History for the material collected at Havana, Illinois in 1920. Material collected in earlier years at Havana, and at Douglas Lake, Michigan, as well as that from various other places, is in the collection of Professor Frank Smith. The writer wishes to express her appreciation for the use of this material and for other material which he has recently collected. She is further indebted to Professor Smith for his suggestions and interest during the progress of this study.

II. Specific Characters

Sparganophilus eiseni is a rather slender earthworm, which varies in length from 80 mm. to 200 mm. Setae *c* and *d* are in the dorsal half of the worm. The clitellum extends approximately from somites 15-25; the tubercula pubertatis, from 17-22. The spermiducal pores are on somite 19, and the oviducal pores on 14, but both are inconspicuous; the spermathecal pores are just ventrad of the seta line *c*, in the intersegmental grooves 6/7, 7/8 and 8/9. There is no trace of gizzard or of calciferous glands in the digestive tract. Moniliform hearts are present in somites 7-11. The spermathecae are in somites 7, 8 and 9; the ovaries, in 13; the ovisacs, in 14; the spermaries and spermiducal funnels, in 10 and 11. The sperm ducts pass through the longitudinal muscular layer of the body wall in somites 11 and 12, and, from there to the pores, lie in the circular muscular layer, or between it and the epidermis. The lobed sperm sacs are paired and are in somites 11 and 12. The first typical nephridia are in somite 13 or 15. Accessory reproductive glands may be present in some one or more of somites 3 to 10, and are regularly present in 23-26 or adjacent somites. Both worms and cocoons are figured by Smith (1915).

III. Material and Its Preparation

Material for this study was collected between July 1919 and February 1921, in the region of Douglas Lake, Michigan, and at Homer Park and Havana, Illinois. The collecting in the former locality was done while the writer was in attendance at the University of Michigan Biological Station, which is on the shore of Douglas Lake. Although search was made for Sparganophilus at many points along the shores of Douglas Lake and of its tributaries, specimens were found only in six places. These places are near, and perhaps have been a part of the shore line at some past time, or are now connected with the lake shore. They are supplied with decaying organic matter but are separated from each other by stretches of shore, parts of which are bare sand. Two mature worms were collected in one spot on the northwest shore of the Lake, but since they were the only ones in the vicinity, they may have been carried there from some other place. Large numbers of worms were found in Diogenes Pond, at Hook Point, in

Sedge Pond, in the banks of Bessey Creek, and in the banks of Maple River. Maple River flows out from Douglas Lake, and, after a long and tortuous course, empties into Burt Lake which at the nearest point is less than two miles from Douglas Lake. One specimen was brought in with some plants collected by Doctor F. C. Gates at the mouth of this River (Burt Lake). At the entrance of Carp Creek into Burt Lake, specimens of Sparganophilus were abundant. Two unidentified lumbricids were also found here. This was the only place at which the writer collected another species of earthworm with *Sparganophilus eiseni*. At Homer Park, Illinois, specimens of *S. eiseni* have been collected in May, June, August, September, October, December and February. They live along the bank and in the bed of a small stream, Salt Fork, sometimes among gravel and small stones, sometimes in soft, mucky soil.

In all these places in which worms were collected, decaying organic matter was present. They were never more than 12 to 18 inches from the edge of the water, and as the water gradually receded in Sedge Pond, the fresh castings were always close to its edge. The worms were usually found at a depth of one to four inches. Frequently both worms and cocoons were found among the roots of grasses growing in or near the water.

The first step in preparing the worms for study was to clean out the digestive tract. This was accomplished by keeping the worms in a vessel with wet filter paper or cloth for 36 to 48 hours. They were then anesthetized with chloretone solution, straightened out between sticks and killed. A solution of alcohol and formalin; a saturated solution of corrosive sublimate; and a solution of corrosive sublimate and acetic acid were used as killing fluids. The latter was used exclusively for the smaller embryos. Sections were stained with Ehrlich's haematoxylin, and a few were counterstained with eosin or orange G.

IV. Development

(1) Observations on the Cocoons and Living Embryos

The cocoons of *S. eiseni* are elongated, but not all of them are as slender and long as those figured by Smith (1915, figs. 8 and 9). The formation of cocoons evidently occurs chiefly during July and August, and to a limited extent during the last half of June and the first two weeks of September. When first formed the cocoons are soft, colorless and transparent or semi-transparent. They are enclosed in a slime tube which may be two or three times as long as the cocoon. The cocoons gradually assume a straw color, become less flexible, and lose the slime tube, probably within six hours. They usually remain sufficiently transparent so that the eggs within them are visible. The cocoons which were formed after the worms had been in the laboratory (in dishes with wet filter paper) for three or

four days were misshapen and opaque in comparison with those which were formed during the first day or two in the laboratory, or those which were found in their natural environment. Although it is not uncommon to find eight or ten eggs in a cocoon, the number of fairly well developed embryos is usually one to four. Seven such embryos have been found in one cocoon, and five or six in each of several cocoons. From a large number of cocoons, perhaps 25% of those which were brought into the laboratory and were apparently normal, worms did not emerge. Of those cocoons from which worms failed to hatch, some remained perfectly transparent, and some became more or less opaque. Many of the latter, when opened, contained disintegrated embryos. In some of both transparent and opaque cocoons, but chiefly the latter, ciliates were found. Some of these cocoons were not tightly sealed, for a slight pressure caused them to emit their contents. Ciliates from the surrounding water might have invaded such cocoons. However, other cocoons were so tightly sealed that they had to be cut open, and the ciliates were seen in the albumen as it was forced out of the cocoon. In a few instances rhabdocoels, rotifers and nematodes were apparently in the cocoons. Beddard (1892) reported finding nematodes in the cocoons of *Octochaetus* (originally *Acanthodrilus*) *multiporus*.

From two cocoons which were brought into the laboratory when the eggs were in early cleavage or the blastula stage, worms hatched out in 24 and 26 days, respectively. Other cocoons were kept for 33 days before the worms emerged. Empty cocoons are open only at one end. Some of the worms, at hatching, are twice the size of others. Such a difference in size is found frequently, when there are two to six embryos in one cocoon. Again one cocoon may contain eggs in cleavage stages and also well formed gastrulae. It scarcely seems that the difference in the time of fertilization would be great enough to cause such a difference in size. Possibly there are periods of cessation of development, or variations in the rate of metabolism.

Four bifid embryos have been taken from cocoons. Each has a single anterior part and two posterior ends. Two of these embryos were the complete contents of one cocoon and were removed before they had attained the degree of development which usually occurs within the cocoon. The third was noticed in a cocoon which had been in the laboratory 34 days. It was moving actively, but, although the opening at the end of the cocoon was enlarged, it did not escape. After eight more days the posterior ends began to disintegrate and it was removed and fixed. A study of these embryos shows a type of dorsal union. A single anterior digestive tract bifurcates into the two digestive tracts of the posterior ends. On opposite sides of the single digestive tract and continuing directly posteriad into each branch, is a nerve cord with four pairs of setae in the

normal relative position. The anterior portions contain from 7 to 20 or 25 somites, and equal from one-seventh to three-fifths of the total lengths.

One bifurcated worm which had probably been out of the cocoon only a few days was found in debris which had been brought into the laboratory. An unsuccessful attempt to keep it alive resulted in the disintegration and loss of the bifurcated portion. Figure 1 shows the manner of bifurcation, which was posterior and which extended through about one-fifth of the total length. The branch was somewhat shorter than the end which was in line with the main axis, but, like it, had an anus, a growing region, a ventral vessel and a pulsating dorsal vessel.

(2) Embryology

The study of the embryology of *Sparganophilus eiseni* is incomplete, but because of the difference of opinion in regard to several points in the embryology of earthworms, it seems best to mention the more important facts noted. Wilson described the following parts in the germ bands of Lumbricus: a thin outer layer of ectoderm; a middle layer formed from four pairs of large cells which are known as teloblasts and which are ectodermal in origin; and an inner layer of mesoderm formed from large cells which are known as mesoblasts. Two large mesoblasts with the mesodermal bands extending forward from them are present in embryos of *Sparganophilus eiseni*. The teloblasts are somewhat anterior to the mesoblasts and are less readily identified. In sections of 0.8 mm. and 1 mm. embryos they form a part of the surface ectoderm, but in 2 mm. embryos they are more or less sunken beneath the ectoderm.

Continuous with and anterior to each teloblast is a row of cells to which it has given rise. Because of the structures which develop from these rows the median or first pair of rows are known as the neural rows, and the second as the nephridial. Staff attributed the formation of the circular muscular layer of the body wall to the third and fourth rows. Earlier investigators (Wilson, Bourne 1894a) had not reached the same conclusion. In longitudinal sections of the 0.8 mm. embryo of *S. eiseni*, the neural rows are covered by a thin cellular layer and can be traced to the sides of the mouth, but the second and third rows are at the surface and can be traced forward through only a comparatively few sections. Even in sections of a 2 mm. embryo the third and fourth rows are lost at times. In whole mounts of 2 mm. to 3 mm. embryos the four pairs of rows show definitely. The first pair are closely approximated in the midventral line; the second row shows the heavy masses or coils of the early nephridia; the third row is a straight line in which no structural differentiation shows; and the fourth row is a series of oval bodies, one in each segment. The development was not followed further, but the difference in the appearance of the third

and fourth rows would scarcely indicate that they form the same adult structure, namely, circular muscles.

V. Morphology

(1) External Characters

There is a wide variation in the size of the mature specimens of *S. eiseni* collected. The largest are over 200 mm. in length and 2.6 mm. in diameter, and the smallest are only 80 mm. to 100 mm. in length and about 1 mm. in diameter. The former are from certain places in the vicinity of Douglas Lake, Michigan, and the latter from Havana, Illinois. In these places there are also worms of intermediate size. No regular difference other than size has been noted among these worms.

The clitellum begins dorsally on somite 15 but may not include all of that somite. The posterior end of the clitellum is somewhat variable. There is usually a gradual decrease in thickness, beginning on 24 and sometimes extending onto 26. Ventrally the clitellum is thin, and scarcely distinguishable except in sections. This ventral part of the clitellum extends from 14-26 or 27, and is usually somewhat thicker in the region of 22 and 26. On worms, which were killed in the manner described, the clitellar region is definitely enlarged; but in some specimens which were apparently put directly into formalin or alcohol, it is no greater in diameter than the somites adjacent to it. It is, however, distinct because of a slightly darker color and because of the absence of intersegmental grooves.

The tubercula pubertatis seem to be typically on somites 17-22, but may extend onto 16 and 23. A specimen from Wisconsin has the tubercula pubertatis on 18-22, and two from Homer Park, Illinois, in which the clitella and tubercula pubertatis are developing, have the latter on somites 18-22.

Ventrally the clitellar somites are flattened and the tubercula pubertatis form longitudinal ridges along the lateral edges of part of the flattened area. The flattened area usually extends posteriad onto 26 or 27, which extent is one or two somites posteriad of the dorsal clitellar thickening. Sometimes a pair of narrow ridges extend posteriad from the tubercula pubertatis, along the lateral edge of this flattened area and border its rounded posterior end. The posterior end is always rounded and the whole flattened area definitely outlined, when the clitellum is well developed, even if the ridges are not present. Narrow ridges also extend anteriad from the tubercula pubertatis in some specimens. The tubercula pubertatis do infringe on the width of the flattened area between them, but in neither the complete ventral flattening, nor the anterior part of it, has the writer noted in any specimen the hour-glass shape which was mentioned by Eisen (1896) as occurring in *S. eiseni*.

(2) Pharyngeal glands

The term, pharyngeal glands, is used in this discussion for those masses of deeply staining cells, which are associated with the muscles extending from the pharynx to the body wall of *S. eiseni*. In somites 5, 6 and 7 the cells are aggregated in masses which are attached to the large pharyngeal muscles extending through those somites. The septum 3/4 is incomplete and the cells of this region are scattered singly and in small groups between the muscles, near their attachment to the pharynx. Eisen (1896) called the latter salivary or suprapharyngeal glands, and the former septal or intestinal glands. He described ducts, which open into the pharynx, from both kinds of glands. The writer has not found such ducts in *S. eiseni*. Stephenson (1917) was unable to find any ducts from similar glands in several species of Pheretima and of Helodrilus. He concluded, from his studies, that both pharyngeal and septal glands were of peritoneal origin, and were not related to the pharynx in the manner originally supposed. The name, chromophil cells, was suggested by him for these structures. It seems to the writer that it would be best to retain the name, pharyngeal, until the origin of these cells from some source other than the pharyngeal wall is definitely established. The term gland is used for convenience.

Eisen stated that the glands of somite 6 were as large as those of 5 in some species, but smaller in others. Table IV (p. 24) shows that the relative size of the glands of 6 is variable, and that glands are sometimes present in somites 7 and 8. Except in the immature specimens, the glands of 6 are usually somewhat smaller than those of 5, in worms which were collected in Michigan. In some of the specimens collected at Homer Park, Illinois, the glands in 6 are as large as those in 5, and in others, they are smaller. The writer could find no fixed relation between the presence of the anterior accessory reproductive glands and the size of the pharyngeal glands of 6.

It has been suggested that such pharyngeal glands are homologues of the nephridia in certain worms in which the latter are absent in the anterior somites. The subsequent discussion of the development and disintegration of the anterior nephridia (p. 12) and the theory (Hesse) of the origin of the pharyngeal glands from the pharyngeal wall would both tend to contradict the suggestion.

(3) Digestive System

The digestive tract has neither gizzard, esophageal glands, nor typhlosole. A dorsal sac opens into the pharynx and the inner surface of the esophagus usually has numerous irregular, but chiefly transverse folds in somites 4 and 5. In the succeeding somites it is folded longitudinally. In 9 or 10 and one or more succeeding somites the diameter of the digestive

canal is frequently greater, and the thickness of the walls is less than in the somites immediately anteriad or posteriad. These facts indicate that the enlargement is a temporary expansion. Since the perienteric blood sinus begins in somite 9, the intestine may be said to begin in 9, although no other structural difference has been found by which to distinguish esophagus and intestine.

(4) Vascular System

The main vessels of the vascular system of *S. tamesis* and *S. eiseni* are similar. They have been described by Benham (1893) and Smith (1895), respectively. Eisen (1896) added the description of the blood glands, but he was in error in stating that the hearts are in somites 8 to 11 in *S. eiseni*. In all specimens studied, including one identified by Eisen as *S. benhami*, the hearts are in somites 7-11. The hearts decrease in size from posterior to anterior, but all five pairs can be seen pulsating in living worms. The hearts do not contract simultaneously, but quickly and in rapid succession beginning with the most posterior one. It is evident that the condition of the hearts at the time when the worms were killed (whether they had just contracted, or were fully expanded) would make a difference in their size. Among the worms studied, the hearts are all moniliform, but in some they are more contorted and of relatively greater diameter.

(5) Excretory System

(a) *Structure of a Nephridium*

The excretory organs of *S. eiseni* are paired meganephridia which open to the exterior through pores placed anteriad of the ventral setae. These nephridia are large, compact organs, consisting of a lobed mass of cœlomic epithelium in which the nephric tubule is embedded. Similar nephridia have been described in other species of Sparganophilus and in other genera. They present an appearance quite different from that of the nephridia of Lumbricus (Benham 1891), of *Maoridrilus rosae* (Cameron) or of the widely distributed *Helodrilus caliginosus trapezoides*. In the latter a nephridium does not appear as a mass of tissue but as a series of loops or a group of convoluted tubules. The tubule, however, is the essential part in both types of nephridia.

The nephridia of *Sparganophilus eiseni* are so compact that tracing the complete course of the tubule in the entire nephridium is impracticable, if not impossible. Tracing the tubule in sectioned nephridia is equally difficult. Of several nephridia which were dissected out, stained in Delafield's haematoxylin and cleared in glycerine, one was sufficiently spread out to trace the greater part of the course of the tubule. The nephridium was then sectioned, approximately in the plane outlined.

The single line in figure 2 represents the general course of the tubule as it was worked out from the study of the whole mount and the sections. The septum, nephrostome and duct connecting the latter with the nephridial mass were torn off, and are consequently shown by broken lines. The break (*br*) was caused by displacing the lobe from its position over the body of the nephridium. Aside from these interruptions the diagram shows an unbranched and continuous tubule from the nephrostome to the nephridiopore. Most of it is in two long and somewhat convoluted loops.

Each nephridium, as is generally true, consists of a pre-septal and a post-septal part. The former includes a nephrostome or funnel and a short duct. The funnel is really a broad tube with two extensions, a large lip and, opposite it, a very small lip. In the large lip the large marginal cells and, outside of them, a row of extra-marginal cells are distinctly visible from the front or inner side of the lip. When this lip is sectioned, there is a noticeable thickening of the edge produced by one or two additional rows of extra-marginal cells, which curve over the edge onto the outside of the lip. The smaller lip is merely a slight extension of the cells of the tube and presents no special structures. Flattened cœlomic epithelium covers the outside of the entire funnel and continues over the duct. The broad tube which forms the body of the funnel gradually narrows into the duct which connects the nephrostome with the nephridial mass. The cell walls are not visible, but judging by the number and position of the nuclei, the tubular portion of the funnel is made up of two series of cells. Just anterior to the septum the number of nuclei indicates that a single row of perforated cells forms the duct. There is probably a gradual change from the intercellular to the intracellular condition, as the funnel narrows into the duct.

There is nothing to indicate that the post-septal part of the nephridium is not intracellular throughout its length. It may be distinguished, according to its structure, into three parts which are indicated in figure 2. They are the narrow tube (*nar*) which forms the first loop; the middle tube (*mid*) which extends to the apex of the second loop; and the wide tube (*wi*) which extends from the apex of the second loop to the nephridiopore (*ne*). The narrow tube is slender and thin-walled. Parts of it are ciliated. The middle tube has a thicker wall and is ciliated throughout its length. The first part of this tube is coarsely granular. Somewhat beyond the middle the coarse granules gradually disappear and scattered groups of fine granules appear. The fine granules increase in number and become aggregated so that in the last of the middle tube they form a layer near the middle of the wall of the tube (fig. 9). The granules are arranged rather regularly in closely packed radial rows, with occasional strings of granules extending out into the peripheral portion of the wall. In some nephridia

these strings are sufficiently numerous and branched to form a network. This layer of granules stains differently from other parts of the nephridium and is distinct in all nephridia.

A constriction separates the middle and wide tubes (fig. 9). The wide tube is without cilia in all parts but the structure of the wall is not the same throughout. A short portion, the ampullar region, at the beginning of the wide tube is made up of rather large deeply stained and definitely outlined cells. Both cross and longitudinal sections of this portion show very distinctly the intracellular nature of the nephric tubule. The first third or half of this region is an enlargement, the ampulla (*am*), which contains deeply staining masses in its lumen. The sectioned walls show a darker inner and a lighter peripheral cytoplasm. In the peripheral cytoplasm there is a network of granules which is similar to that in the peripheral cytoplasm of the middle tube, and which seems to condense into the darker inner cytoplasm. The inner cytoplasm differs from the layer in the middle tube in that it lacks the radial striations and stains less intensely. The distinction between inner and peripheral cytoplasm gradually disappears in the large cells beyond the ampulla. These large cells abruptly give place to a thin-walled tube in which the cell boundaries are indistinguishable. The last part of the wide tube (fig. 5) begins with cells similar to those of the ampullar region, but in its distal part the wall becomes thinner and the cell boundaries gradually disappear. Figure 7 represents the distal part of the wide tube, including that which passes through the body wall to the epidermal[1] invagination, in a nephridium which had been dissected out and sectioned. The number of nuclei in this and adjacent sections of the tube indicates that it is intracellular. Figure 8 shows a section of the complete epidermal invagination of the same nephridium. Since adjacent sections show no cellular structure other than that which is shown in these two figures, it is evident that the wide tube opens directly into the nephridiopore, and that there is no muscular duct. Serial sections of nephridia *in situ* substantiate this conclusion.

The shape of the nephridium and position of the different parts of the tubule within it are variable. However, the two long loops are regularly approximately parallel, and for the greater part of their length, are embedded in large lightly staining cells (fig. 6). These large cells are usually found about the periphery rather than in the central part of the nephridium, and they have not been noted except in relation to the nephric tubule. These facts suggest that the large cells may have a direct relation to the process of excretion.

[1] The outer cellular layer covering the earthworm has been called epidermis, and also hypodermis. Neither name is satisfactory, but because of its more general usage the former is used here.

The smaller, evenly granular cells, which form the nephridial mass, are probably epithelial. The septa are covered by delicately stained projections, some large, some small and closely crowded; some with nuclei, others without nuclei. These projections are parts of vesicular epithelial cells. Similar projections, without nuclei, cover the surface of the nephridium. These projections are frequently found to be parts of the evenly granular cells of the nephridial mass. Evidently, then, these smaller cells in the nephridial mass are epithelial cells, but only those at the surface are vesicular.

The structure of the nephridial tubule of Lumbricus has been described by Gegenbauer, Benham (1891) and Maziarski (1905). The last paper is available only in the form of a review by Meisenheimer. K. C. Schneider studied the nephridium of *Eisenia rosea*, one of the Lumbricidae, and Cameron, the nephridium of *Maoridrilus rosae*, one of the Megascolecidae. The nephridium of each of these has three parts similar to those of *Sparganophilus eiseni*, and in addition a muscular duct which is located between the wide tube and the pore. Intermittent ciliation of the narrow tube, complete ciliation of the middle tube and lack of ciliation in the wide tube are characters common to all. Histologically there are differences in the structure of corresponding parts of the tubule. None, except *S. eiseni*, have the more deeply staining layer in the wall of the middle tube. The figure by Maziarski (Meisenheimer) shows some variation in the wide tube, but it is not the same as that found in *S. eiseni*. The nephridia of *Moniligaster grandis* (Bourne 1894b), and of *Perieodrilus ricardi* and *P. montanus* (Benham and Cameron) are distinctly different in structure from those of the above mentioned earthworms.

From the foregoing discussion it appears that although there is a general agreement between the nephric tubule of *Sparganophilus eiseni* and that of Lumbricus, from the nephrostome to the muscular duct, the former has a higher degree of specialization in the middle and wide tubes.

(b) *History of the Anterior Nephridia*

Sparganophilus is one of several genera of earthworms which resemble certain limicoline forms in lacking nephridia in the anterior somites. Smith (1895) and Eisen (1896) both listed somite 13 as the first one containing nephridia in *S. eiseni*. In other species the first nephridia have been recorded in 12, 13 and 16. However, a study of embryos shows that nephridia are present in some of the somites anterior to 13 (Table I). The first distinct nephridia are in somite 3 of seven embryos; in somite 4 of five embryos; in somite 6 of four embryos and in somite 12 of two young worms. Unfortunately no first nephridia have been found in somites 9, 10 and 11, and only one in each of somites 7 and 8. In addition to the fact that, generally in the larger embryos, the nephridia are absent in the more

anterior somites, there are signs of disintegration of the anterior nephridia. The first nephridia are called distinct rather than well developed or typical because, while there can be no doubt that they are nephridia, the canal in some is indistinct if visible at all. This must be due to disintegration for the canal develops very early as is shown by its presence in figure 11, which is typical of the nephridia of three successive somites, probably 3, 4 and 5, of a 0.5mm. embryo. Figures 10 and 12, from sections through nephridia of somite 3 of embryo No. 2 and of somite 5 of embryo No. 7, respectively, show very distinct canals; but in figure 13 from a section through one of the nephridia of somite 3 of embryo No. 7, only one indistinct section of the canal is recognizable. The canal shows more definitely in this section than in any other sections of the nephridia of this somite.

TABLE I

FIRST NEPHRIDIA IN EMBRYOS AND VERY YOUNG WORMS

Nos. 20 and 21 are the same as 99a and 100a of Table II, respectively.

Number	*Length*	*Somite*	*Number*	*Length*	*Somite*
1	1 mm.	3	12	15 mm.	4
2	1.5 mm.	3	13	8 mm.	5
3	2 mm.	3	14	5-6 mm.	6
4	3-4 mm.	3	15	.....	6
5	4-5 mm.	3	16	7-8 mm.	6
6	.....	3	17	10 mm.	6
7	7 mm.	3	18	.....	7
8	4 mm.	4	19	8 mm.	8
9	4.5 mm.	4	20	17 mm. worm	12
10	5 mm.	4	21	15 mm. worm	12
11	6 mm.	4	..	.....	..

Other first or first and second nephridia show similar conditions and in somites anterior to the first nephridia there are frequently small masses of tissue which are located and stained similarly to nephridia. These are evidently rudiments of nephridia. They are in somite 3 of Nos. 9, 10, 11 and 12, and in one to three somites anterior to the first nephridia of Nos. 13 to 20. In somite 2 of several embryos there are small masses of tissue which may be nephridial rudiments, but no nephridia, as distinct as the smallest of those of somite 3, have been definitely located in somite 2. All these facts indicate that the nephridia develop in the anterior somites and then disintegrate in an antero-posterior order. If the number of somites rather than the length of the embryo had been used as a measure of relative development, there might be less variation among those worms which have the first nephridia in 3, 4 or 6. Part of the variation in size is doubtless due to the degree of contraction or relaxation of the embryos. However, such

a marked divergence as is shown by Nos. 7, 12 and 19 seems to indicate variations in the rate of disintegration of the nephridia. Possibly such variations are correlated with differences in the rate of metabolism, which differences, as was suggested above, may be related to the distinct differences in size frequently found among embryos in the same cocoon.

The development of the nephridia takes place in an antero-posterior direction. Each nephridium is consequently visibly farther advanced than those from eight to ten somites posteriad of it. But, since the rate of development is more rapid than that of disintegration, the nephridia of somites 10-12 reach a more advanced stage of development than do those of somites 3-6. In No. 14 the nephridia of somite 7 have more convolutions and a canal of proportionately greater diameter than do the nephridia of somite 3. In somites 7 and 8 of No. 18, large cells, similar to those in which part of the nephric tubule of the adult is embedded, can be recognized. In No. 20 the epithelial masses have developed about the nephridia of 12. The development of the nephrostome has not been followed, but there are funnels in 9 and some of the succeeding somites of No. 19, and in several somites of No. 18. The nephridiopores are readily recognizable in somite 8 of No. 19 and in somites 7-10 of No. 18, but not in other worms in which the nephridia extend as far forward as somites 7 and 8. In specimens in which typical nephridia are present in 12 the pores are distinct in that somite.

A study of mature and immature worms discloses a variation in the position of the first typical nephridia. Table II shows the condition of the nephridia in those worms in which they were studied. Table III is a summary of Table II. A typical nephridium is one, such as has been described, with its tubule embedded in a mass of cells, and having a definite nephrostome and nephridiopore. The reduced nephridia vary from a small mass of epithelial cells without a tubule to a mass about the size of a normal nephridium, but with reduced tubule and without a nephrostome or a nephridiopore. The typical and reduced nephridia, especially of 13, grade into each other so that it is sometimes difficult to distinguish them. This is because the disintegration of the nephrostomes is variable, and the nephridiopores may persist and retain an indistinct connection with a nephridium in which the tubule is reduced. Table III indicates that reduced and typical nephridia are present in increasing numbers in somites 11, 12, 13 and 15, and that all nephridia in 15 are typical. All nephridia in somites posteriad of 15 are also typical.

The increasing number of nephridia in somites 11, 12, 13 and 15 is doubtless due to the antero-posterior order of disintegration. The different conditions of the nephridia of 12, of 13 or of 14 in different individuals of *S. eiseni* are probably due to individual variations. It is possible that the nephridia of these somites are reduced increasingly with age or successive breeding seasons; but, since no method has been found for

distinguishing worms of different ages, there is no evidence for such a progressive reduction. On the other hand, individual variations have been noted in the nephridial condition of embryos. Specimen No. 101a

TABLE II

NEPHRIDIA OF YOUNG AND ADULT WORMS

t indicates a typical organ: *s*, a reduced organ: *o* and a blank, absence of the organ. Two symbols are used when the nephridia of the two sides are dissimilar. Identical specimens have the same number as in Table IV.

No. of specimen	*Size of worm or clitellum*	*Nephridia in somites*					*No. of specimen*	*Size of worm or clitellum*	*Nephridia in somites*			
		11	12	13	14	15			12	13	14	15
15	*t*			*t*	*s*	*t*	100	35 mm.	*t*	*t*	*t*	*t*
22	*t*			*t*	*o*	*t*	101	35 mm.	*so*	*t*	*t*	*t*
31	*t*			*t*	*s*	*t*	101a	17 mm.		*to*	*t*	*t*
31a	*t*			*s*	*o*	*t*	102	immature	*s*	*t*	*t*	*t*
33	*o*			*t*	*s*	*t*	103	*o*		*to*	*o*	*t*
34	*t*			*t*	*s*	*t*	104	*s*		*ts*	*o*	*t*
35	*t*			*s*	*s*	*t*	105	*s*		*t*	*o*	*t*
36	*t*			*s*	*s*	*t*	106	*t*		*t*	*o*	*t*
37	*t*		*s*	*t*	*s*	*t*	107	*t*		*t*	*s*	*t*
38	*t*		*s*	*t*	*s*	*t*	108	*t*		*t*	*so*	*t*
40	*o*			*t*	*s*	*t*	109	*t*		*t*	*so*	*t*
41	*s*			*t*	*s*	*t*	110	*t*		*to*	*o*	*t*
42	*s*			*t*	*s*	*t*	111	*t*		*t*	*o*	*t*
43	*o*		*s*	*t*	*s*	*t*	112	*t*		*t*	*o*	*t*
44	*o*		*s*	*t*	*s*	*t*	113	*t*		*t*	*o*	*t*
45	*s*		*s*	*t*	*s*	*t*	114	*o*	*s*	*t*	*s*	*t*
46	*s*			*ts*	*s*	*t*	121	*o*	*s*	*t*	*s*	*t*
52	*s*			*t*	*s*	*t*	122	*o*	*so*	*t*	*s*	*t*
53	*o*		*s*	*t*	*s*	*t*	123	*t*	*s*	*t*	*s*	*t*
54	*o*		*s*	*t*	*s*	*t*	128	*t*		*t*	*o*	*t*
55	*s*		*so*	*t*	*s*	*t*	129	*t*		*so*	*o*	*t*
57	*s*		*s*	*t*	*s*	*t*	138	*t*		*t*	*o*	*t*
58	*o*		*s*	*t*	*s*	*t*	139	*t*		*t*	*s*	*t*
59	*o*		*s*	*t*	*s*	*t*	140	*t*		*s*	*o*	*t*
60	*o*		*s*	*t*	*s*	*t*	141	*t*		*s*	*s*	*t*
83	*s*			*t*	*s*	*t*	142	*t*		*so*	*o*	*t*
84	*t*		*so*	*t*	*s*	*t*	143	*t*		*o*	*o*	*t*
85	*t*		*s*	*t*	*s*	*t*	144	*t*		*o*	*o*	*t*
99	45 mm.	*s*	*s*	*t*	*t*	*t*	145	*t*		*t*	*o*	*t*
99a	17 mm.	*s*	*t*	*t*	*t*	*t*	152	*t*		*s*	*s*	*t*
100a	15 mm.		*t*	*t*	*t*	*t*	155	*t*		*t*	*o*	*t*

shows a marked difference from others of its size. Adult specimens from different localities show greater or lesser tendencies toward the disappearance of nephridia in certain somites.

Other worms in which the most anterior nephridia are in some one of somites 10 to 16 are *Sparganophilus tamesis*, *Criodrilus lacuum*, *Alluroides pordagei*, *Glossoscolex giganteus* (originally *Titanus brasiliensis*), *Haplotaxis heterogyne* and several species of Pontodrilus. The embryonic condition of the anterior nephridia of these is not known. Vejdovský (1888-1892) described the development and subsequent disintegration, during embryonic life, of the nephridia of 1-6 of Rhynchelmis, and mentioned a similar process in Chaetogaster, Aeolosoma and Nais. Bourne (1894a) found that the nephridia of *Diporochaeta* (originally *Perichaeta*) *pellucida* attained a well developed condition in somites 2-6 and then degenerated, while those of 7-11 became complex. All of the worms mentioned, which lack nephridia in ten or more anterior somites, live in or at the edge of the water.

Bage and Stephenson (1915) found that, in certain Megascolecidae, micronephridia are present in all somites of the body, and that in the posterior somites, sometimes as far forward as 12, there are also meganephridia.

TABLE III

SUMMARY OF TABLE II

All with nephridia *ts* or *to* are counted with *t*.
All with nephridia *so* are counted with *s*.

		Nephridia		
		o	*s*	*t*
Somite 11	Mature	55	0	0
	Immature	5	2	0
Somite 12	Mature	37	18	0
	Immature	1	3	3
Somite 13	Mature	2	8	45
	Immature	0	0	7
Somite 14	Mature	19	36	0
	Immature	0	0	7
Somite 15	Mature	0	0	55
	Immature	0	0	7

Still other species of earthworms have a thick cœlomic covering on the posterior but not on the anterior nephridia, or the muscular duct may be more highly developed in one part of the body than in another. In a few species the nephridia are larger in a few anterior somites than in the posterior ones. Such modifications are similar to the absence of nephridia in the anterior somites.

It has been thought that the absence of anterior nephridia in earthworms is correlated in some way with the reproductive system. However, disintegration of the nephridia of *Sparganophilus eiseni* begins before there is any trace of the reproductive organs. The gonads, which are the first parts of the reproductive system to develop (p. 17), are not recognizable until the nephridia of somite 8 are beginning to disintegrate (No. 19). Furthermore, if the nephridia disintegrated as the reproductive organs developed, they would disappear not in an antero-posterior sequence, but first in somites 10, 11, 13; then in 11, 12, 14; lastly in 7, 8, 9; and probably not at all in the somites anteriad of 7, since there are no reproductive organs in those somites. The probable influence of the genital ducts on the nephridia of 11, 12 and 14 will receive attention in the discussion of the genital ducts. The time and order of the disintegration of the nephridia of 3-10 indicate that this process is not directly related to the development of the reproductive organs. The adaptation to the aquatic habitat or some other factor may have produced a physiological condition which is different from that in most earthworms and which has resulted in the loss of the anterior nephridia.

(6) Reproductive System

(a) *Time of Development*

The development of the reproductive organs begins about the time that the worms emerge from the cocoons, and is not closely related to the size of the worms. The gonads appear first; then the spermiducal funnels, sperm ducts and sperm sacs develop in quick succession. The oviducal funnels, oviducts and ovisacs develop later, only a short time before the accessory glands and spermathecae which develop as the worm approaches adult size. Finally, with the approach of the breeding season, the clitellum appears. Specimen No. 19 of Table I is the youngest specimen in which the gonads can be identified, and in that only on one side. Nos. 20 and 21 are the same as Nos. 99a and 100a of Table II, respectively. These two and the other immature worms, Nos. 99 to 102, inclusive, (Table II) have distinct spermaries and ovaries. Nos. 99, 101a, 101 and 102 have anlagen of the spermiducal funnels, and the latter three, of the sperm sacs and sperm ducts also. Tiny anlagen probably of the oviducal funnels, are present in Nos. 101 and 102, and definite anlagen of these funnels are present in a 55mm. worm which is not included in the tables. In Nos. 121 and 122, which have reached adult size but probably not sexual maturity, the oviducal funnels are still small and the anlagen of the oviducts are recognizable. Accessory reproductive glands and spermathecae are not developing in any of these specimens except Nos. 121 and 122.

Bergh (1886) found that the spermaries and ovaries were the only parts of the reproductive system of Lumbricus, which developed during embryonic life. In *Octochaetus* (originally *Acanthodrilus*) *multiporus*, Beddard (1892) found, in addition to the gonads, prominent genital funnels in somites 10-13 of embryos that were not yet ready to emerge from the cocoon.

(b) *Genital Funnels and Ducts*

The anlagen of the genital funnels first appear as deeply staining areas on the anterior faces of septa 10/11, 11/12 and 13/14, just laterad of the nerve cord and near the point at which the nephridial tube penetrates the septum. This area thickens into a mass with deep indentations, from which a deeply staining strand of tissue can be traced through the septum, and toward or into the body wall of the following somite at the point where the nephridium (if present) penetrates to the exterior. This strand is the anlage of the genital duct. It enlarges and as sexual maturity approaches, acquires a lumen. The oviduct opens directly to the exterior in 14, but the sperm ducts pass into the body wall in 11 and 12 and then turn posteriad; those of each side unite and extend, in the body wall, to the spermiducal pores on somite 19. An early stage in the development of the longitudinal ducts has been found in a worm in which the genital funnels are not of maximum size, and the spermathecae and accessory glands are very small. In longitudinal sections the duct appears as two narrow parallel bands in the longitudinal muscular layer. In each band are many nuclei, while numerous similar nuclei are located close to the duct and scattered through the longitudinal muscular layer. Since the normal position of the longitudinal part of the sperm duct in the adult is between the epidermis and the circular muscular layer or in the latter, there must be a shifting of this duct as it develops.

No difference has been noted in the development of the spermiducal and oviducal funnels or in the ducts from them to the body wall. The mature spermiducal funnel is much larger than the oviducal funnel, but sections show that the difference is one of size rather than structure. Between periods of breeding activity the funnels revert to small solid masses and the ducts to solid strands of tissue.

Although the nephridia degenerate more or less completely before the genital funnels develop, there is a limited amount of evidence in regard to the morphological relations of excretory and reproductive systems. The genital funnel anlagen first appear as parts of the epithelium of the septa, while the nephridial funnels, at all stages seen, are separated from the septa by the short pre-septal portions of the ducts. In Nos. 101 and 102, the nephridial funnels are still typical in somite 13, and there are also small deeply stained areas which are probably the anlagen of the oviducal fun-

nels. On one side of somite 13 of No. 122 there is a small mass of tissue, which has the shape of a nephridial funnel, and which is in line with the nephridial funnels of 12 and 14. It is probably the remnant of the nephridial funnel, and laterad of it is the very definite anlage of the oviducal funnel. From the latter, the anlage of the oviduct can be traced through the septum and toward the body wall. These facts indicate that the genital funnels are not derived from the nephridial funnels. The development of the genital ducts from the genital funnels has been described above. The oviduct passes into the body wall close beside the nephridium and opens to the exterior through an epidermal invagination, which, if one may judge by its position, is the nephridiopore. The sperm ducts likewise pass into the body wall close beside the nephridia, but they open to the exterior on somite 19, and entirely independent of the nephridia.

Vejdovský (1884) described the anlagen of the genital funnels as thickenings of the septa in Naididae, Enchytraeidae, Tubificidae and Lumbriculidae. Bergh (1886) found the same type of development in Lumbricidae. In *Tubifex rivulorum*, Gatenby found a difference in the development of spermiducal and oviducal funnels, but both developed from cœlomic epithelium. All these investigators found that the genital ducts developed as outgrowths from the genital funnels.

Stolc (Beddard 1892) reported that in Aeolosoma the spermatozoa passed out by way of the nephridia, especially those of somite 6, and that during the sexual period the nephridia of certain somites disappeared wholly or in part. In Marionina sp. (originally *Enchytraeoides marioni*) Roule found that the nephridia in 1-8, and in 12 were lacking in early stages of embryos; that the sperm ducts subsequently developed in 12, and that with the attainment of sexual maturity the remaining nephridia of 9-14 disappeared more or less completely. He regarded the sperm ducts as nephridia whose development had been delayed. It seems to the writer more probably that the nephridia of 1-8 and of 12 of Marionina had developed and disintegrated in earlier stages, and that the sperm duct probably originated as Vejdovský described it in Enchytraeidae.

Beddard (1892) concluded that the genital funnels developed from the nephridial funnels, and the genital ducts, from parts of the nephridial ducts in *Octochaetus multiporus*. The conditions in this species are similar to those in *Sparganophilus eiseni*, except that in the latter there is no apparent continuity between the genital and nephridial ducts. Benham (1904) found, in both mature and immature specimens of *Haplotaxis heterogyne*, that the most anterior nephridia were in somite 10 and were somewhat degenerate, and that there were no nephridia in somites 11-13. Because of the marked structural similarity between sperm ducts (in 11 and 12) and nephridia, he concluded that the sperm ducts were nephridia onto which genital funnels had been grafted. Since the oviducts had no resem-

blance to the nephridia, they were considered different in origin. No explanation of the absence of nephridia in 13 was offered.

Reduction of the nephridia in the genital somites alone was reported in Moniligaster (Benham 1886) and in Gordiodrilus (Beddard 1894). In *Libyodrilus violaceous* Beddard (1891) found nephridia in all somites posterior to 3 in a young specimen; but in the adult all that remained of the nephridia of 13-17 was the integumental network. Beddard attributed the loss of nephridia to the presence of very large spermathecae which extended through these somites.

It has been concluded (p. 17) that the disintegration of the nephridia of somites 3-10 of *Sparganophilus eiseni* is not directly related to the development of the reproductive organs; and (p. 19) that the genital funnels and ducts develop independently of the nephridia, although the same pore may serve first for a nephridium and subsequently for an oviduct. With the exception of definitely immature specimens, none have typical nephridia in 11, 12 and 14, in which somites the genital ducts are located. The nephridia of 11 are merely rudiments or are lacking in those specimens in which the anlagen of the spermiducal funnels are found. Of the immature specimens, Nos. 99a, 100a, and 100 have no spermiducal funnels but do have typical nephridia in 12; Nos. 99, 101, 101a and 102 have the anlagen of spermiducal funnels but have reduced or no nephridia in 12. Nos. 101 and 102 have tiny anlagen of the oviducal funnels and typical nephridia in 14; Nos. 121 and 122 have distinct anlagen of the oviducal funnels and ducts, but the nephridia are reduced. Finally, 45 mature worms have typical nephridia in 13 but reduced or no nephridia in 14 (Table III). Because of these facts, it seems that the disintegration of anterior nephridia which is produced in the embryo by the physiological condition, is augmented in 11, 12 and 14 by the development of the genital funnels and ducts of the respective somites. Since the nephridia are not transformed into genital organs, probably this effect is produced by introducing some different physiological condition, possibly absorption of the food supply or a hormone secretion.

(c) *Sperm Sacs*

The sperm sacs extend into somites 11 and 12 from septa 10/11 and 11/12. During the breeding season, the sacs of 12 are often so large that they push the septum 12/13 back against septum 13/14. Small outgrowths which somewhat resemble the sperm sacs are frequently found on septum 12/13. The sperm sacs have been described as lobulate. Eisen (1896) distinguished between a minutely lobulate condition in *S. eiseni* and a less minutely lobulate condition in *S. benhami*. There is a great variation in the size of the lobules in *S. eiseni* at different seasons. When free from spermatozoa the lobules are quite small, but at the height of

the breeding season, they may be so distended and the whole sperm sac so compact that the lobulation is not readily recognizable. Figures 99 and 119D (Eisen 1896) of *S. benhami* represent conditions frequently found in *S. eiseni*.

(d) *Spermathecae*

Between periods of breeding activity a spermatheca is represented by a small mass of undifferentiated cells. The mass is without a definite lumen, and is placed at the inner end of a more deeply staining strand of tissue which extends through the body wall. The strand is the rudiment of the spermathecal duct.

The spermathecae of the sexually mature worm are variable in shape. Often they are cylindrical with a spherical enlargement at the free end; but the enlargement may be elongated at the expense of the cylindrical portion. Sometimes the contour is smooth, again it is irregular as if the spermatheca had been crowded into a space shorter than the spermatheca itself. Eisen (1896) figured spermathecae of *S. benhami* (figs. 118a and 118b), of *S. carneus* (figs. 140a and 140b), and of *S. guatemalensis* (figs. 141a, 141b and 141c). A specimen, identified by Eisen as *S. benhami*, is in the collection of Professor Smith. It has spermathecae which are slightly irregular in outline, but not so irregular as those figured by Eisen. Some specimens of *S. eiseni*, which have the anterior accessory glands, have spermathecae of the same shape as those of the above mentioned specimen of *S. benhami*. Figures 140a, 140b and 141b (Eisen) are quite typical of the shape of the spermathecae of *S. eiseni*. A sac, similar to the ones which he mentions and figures at the end of the spermathecae of *S. guatemalensis*, has been noted in some specimens of *S. eiseni*.

(e) *Accessory Reproductive Glands*

These are glands which are evidently related to the reproductive activities but are not connected with the sperm ducts or other reproductive organs. There are two kinds, of which one is found in some of somites 3 to 10, and the other in some of somites 15 to 17 and 22 to 27. For the sake of brevity, the first mentioned glands will be called the anterior glands and the last mentioned, the posterior glands, in the following discussion. The posterior glands were called prostates in the original description of the species by Smith (1895). Eisen (1896) followed the nomenclature of Beddard (1895) and applied the term spermiducal to these same glands. Michaelsen preferred to limit the term prostates to the glands which were directly associated with sperm ducts and described the glands of *S. eiseni* as prostate-like. The presence of the anterior glands was first noted by Eisen (1896). He called them parietal glands. This name is in no way suggestive of the function, neither are the posterior glands true prostates.

Consequently, since the anterior and posterior glands are similar in origin and structure, and are related to the reproductive activities, which facts will be shown subsequently, the writer has chosen to use the term, accessory reproductive glands for both kinds, and to distinguish them from each other by the adjectives, anterior and posterior. The term prostates will be used for those glands which are connected with the sperm duct.

Each anterior gland (fig. 4) is a spherical or somewhat elongated mass from which a duct passes through the body wall close beside seta *a* and opens into the follicle of that seta. The glandular part (fig. 16) consists of a tubular lumen surrounded by epithelial, muscular and glandular layers; the duct lacks the glandular layer. The epithelial layer is a continuation of the body epidermis (fig. 18); the muscular, of the circular muscular layer of the body wall. The proximal ends of the glandular cells are attenuated and can be traced into the muscular layer. They probably extend into the epithelial layer.

A posterior gland (fig. 3) consists of a tubular and somewhat convoluted glandular part (fig. 14), and a duct (fig. 15) which opens to the exterior close beside and usually through the follicle of seta *b*. The duct consists of an epithelial layer which is continuous with the epidermis, and a muscular layer. The peritoneum covering the duct is prominent. The glandular part consists of epithelial cells with attenuated processes which extend toward or to the lumen. Because of the regularity of cell walls and of the position of the nuclei about the lumen there appears to be an epithelial lining surrounded by a glandular layer.

The earliest stage in the development of an anterior gland was found in specimen No. 68, which was collected in May and was without a trace of a clitellum. In this gland (fig. 18) the epidermal invagination is distinct and cells from the circular muscular layer can be seen extending part way around it. Figure 19 is a section through the deepest part of the invagination, which is as deep as the thickness of the body wall. In later stages mitotic figures are present in the epithelium. The epithelial nuclei are very numerous and are arranged in two or three irregular series. The muscular layer appears as a thin strand of cells which are not typical muscle cells but which are connected with the circular muscular layer. At this stage the glandular layer first appears. It consists of numerous nuclei and a comparatively small amount of cytoplasm, but is without cell walls. The nuclei are stained like those of the epithelium, are found close to the muscular layer and occasionally are seen in sections directly over the thin muscular strands. Because of these conditions and the attenuated bases of the glandular cells in the fully developed gland, it seems that the glandular cells are epithelial in origin. Nothing has been found which would indicate their development from the body wall or from the cœlomic epithelium. In later stages the epithelial cells become definitely columnar

and arranged in a single layer. The muscular and glandular layers gradually increase in thickness and cell walls appear in the latter. In the development of a posterior gland, which is similar to that of an anterior one, the continuity of the epithelial and glandular cells shows as soon as cell walls appear.

Variations in size and position have been found in both kinds of glands. Table IV gives the condition and location of these glands, in so far as they were ascertained, in the various worms studied. Worms numbered from one to 36 are in the collection of Professor Smith and the remainder have been collected during the course of this study. The specimens from a given locality are listed together in each of the two series. This table shows that, so far as is known, the anterior and posterior glands in each worm are in similar stages of development. Of the worms collected at Homer Park in June and July, all have typical glands; in October, December and February, all have small glands. Furthermore, a distinct clitellum is, without exception, accompanied by typical glands; an imperfectly developed clitellum is most frequently, and an absence of clitellum, in mature worms, is always accompanied by small glands. There is then a decrease in the size of the glands between breeding seasons. This decrease in size is not merely a shrinkage but an actual disintegration and loss of certain layers. Muscular and glandular layers degenerate into a mass (fig. 17, *deg*), in which cell outlines and nuclei are indistinct or wanting, and at the surface of which are numerous bloodvessels. This mass disappears leaving only the epithelial layer, which apparently does not degenerate, for mitosis occurs in its cells both before and after the loss of the other layers. Mitotic figures have been found in the epithelium of worms collected in December, February and May. The subsequent development is similar to that already described. In the posterior glands there is a similar process of degeneration and regeneration.

The original development of these glands is probably not closely related to the breeding season, for in Nos. 94 and 95, which were collected in September, the glands were apparently developing. They were also developing in No. 68 which was collected in May.

The posterior glands were found so regularly in three or four of somites 23-26 in the worms studied first, that, later, only somites 1-15 of most of the worms were sectioned. Sometimes a part of somites 23-26 was sectioned in order to ascertain the condition of the glands. Of all the worms in which the location of the posterior glands has been investigated, only specimens from Havana, Illinois have the glands in somites other than 23-26. Several of these worms have glands in three or four of somites 22-27, and one or more of 15, 16 and 17.

Aside from the seasonal changes, there are in the anterior glands variations in size due chiefly to the thickness of the glandular layer; and vari-

TABLE IV

ACCESSORY REPRODUCTIVE AND PHARYNGEAL GLANDS

Some of the specimens were dissected: from one to 27 somites of the others were sectioned. A blank means that the condition of the organ is unknown. *t* indicates a typical organ: *s*, a small organ: *ss*, a very small organ: *o*, absence of the organ. The numbers which follow these letters indicate the somites.

No. of specimen	*Place or date of collection*	*Clitellum*	*Anterior glands*	*Posterior glands*	*Pharyngeal glands*
1	?		2 *t*, 4		
2	?		*o*		
3	?		*o*		
4	?		*o*		
5	?		*o*		
6	Urbana, Ill.		1 *t*, 8		
7	" "		*o*		
8	?		2 *t*, 6		
9	?		1 *s*, 4		
10	Havana, Ill.		2 *t*, 3 1 *t*, 4		
11	" "		2 *t*, 6		
12	" 1895	*t*	1 *t*, 7	*t*, 16	
13	" 1895	*t*		*t*, 16, 25–27	
14	" Ill.	*t*	2 *t*, 3, 4	*t*	
15	" "	*t*	*o*	*t*	
16	" "	*t*	2 *t*, 4	*t*, 23–26	
17	" "		2 *t*, 3, 4		
18	" "		2 *t*, 3, 4		
19	" "		2 *s*, 3 1 *s*, 4		
20	" "		2 *t*, 3 1 *t*, 4		
21	" "		1, 3, 4		
22	[Diogenes	*t*	1 *t*, 3	*t*	
23	[Pond	*t*	1 *t*, 3		
24	Douglas Lake		2 *s*, 3		
25	" "		2 *t*, 3		
26	" "		1 *t*, 4		
27	" "		1 *t*, 3		
28	" "		2 *t*, 3		
29	" "		1 *t*, 3		
30	" "		2 *t*, 3		
31	Florida	*t*	2 *t*, 4	*t*	
32	"	*t*	2 *t*, 4	*t*	
33	"	*s*	1 *t*, 8	*t*, 23–25	
34	Louisiana	*t*	*o*	*t*, 23–25 23–26	
35	Wisconsin	*t*	*o*	*t*, 23–26	
36	Iowa	*t*	2 *t*, 6	*t*, 24–26	

TABLE IV, continued

No. of specimen	*Date of collection*	*Clitellum*	*Anterior glands*	*Posterior glands*	*Pharyngeal glands*
	Homer Park, Ill.				
37	July 1918	*t*	2 *t*, 4	*t*, 23–26	*t*, 6
38	" "	*t*	1 *t*, 4	*t*, 23–26	*t*, 6
39	Aug. 1919	*t*	*o*	*s*, 23–26	
40	" "	*o*	1 *s*, 4	*s*, 23–26	
41	" "	*s*	2 *t*, 4	*t*	*s*, 6
42	" "	*s*	2 *t*, 4	*t*	*t*, 6
43	" "	*o*	1 *s*, 4	*s*	*s*, 6
44	Oct. 1919	*o*	1 *s*, 4	*s*	*t*, 6
45	" "	*s*	2 *s*, 4	*s*, 23–26	*s*, 6:*ss*,7
46	" "	*s*	1 *s*, 4	*s*	*s*, 6
47	July 1918	*t*	2 *t*, 4		
48	" "	*t*	*o*		
49	" "	*t*	2 *t*, 4		
50	" "	*t*	2 *t*, 4		
51	" "	*t*	2 *t*, 4		
52	May 1920	*s*	*o*	*t*, 23–26	*ss*, 6
53	" "	*o*	2 *s*, 4	*s*, 23–26	*t*, 6
54	" "	*o*	2 *s*, 4	*s*	*s*, 6
55	" "	*s*	1 *s*, 4	*s*, 23–26	*t*, 6
56	" "	*s*	2 *t*, 4		*t*, 6
57	" "	*t*	2 *t*, 4		*s*, 6
58	" "	*o*	2 *s*, 4	*s*	*s*, 6
59	" "	*o*	2 *s*, 4	*s*	*s*, 6
60	" "	*o*	1 *s*, 4		*s*, 6
61	" "	*o*	2 *s*, 4		
62	" "	*o*	1 *s*, 4		
63	" "	*o*	2 *s*, 4		
64	May or June	*t*	1 *t*, 4		
65	June 1920	*t*	2 *t*, 4		
66	" "	*t*	2 *t*, 4		
67	May 1920	*s*	2 *s*, 4		
68	" "	*o*	2 *s*, 4		
69	Oct. 1920	*o*	1 *s*, 4		
70	" "	*o*	2 *s*, 4		
71	Dec. 1920	*o*	1 *s*, 4	*s*, 23–25	
72	" "	*o*	1 *s*, 4		
73	" "	*o*	2 *s*, 4		
74	" "	*o*	*o*	*s*, 23–26	
75	" "	*o*	1 *s*, 4		
76	" "	*o*	2 *s*, 4		
77	" "	*o*	*o*		
78	Feb. 1921	*o*	1 *s*, 4		
79	" "	*o*	1 *s*, 4		
80	" "	*o*	1 *s*, 4		
81	" "	*o*	*o*		
82	" "	*o*	*o*	*s*, 23–26	

TABLE IV, continued

No. of specimen	*Date of collection*	*Clitellum*	*Anterior glands*	*Posterior glands*	*Pharyngeal glands*
	Havana, Illinois.				
83	Sept. 1920	*s*	*o*	*s*, 15–17, 23–26	*ss*, 6
84	" "	*t*	2 *t*, 6		*s*, 6
85	" "	*t*	1 *t*, 6		*s*, 6
86	" "	*t*	1 *t*, 7	*t*, {15–17, 24–27; 16, 24–26}	
87	" "	*t*	2 *t*, 6		
88	" "	*t*	2 *t*, 6	*t*, 23–26	
89	" "	*t*	2 *t*, 6	*t*, 23–25	
90	" "	*t*	*o*	*t*, {23–26; 24–26}	
91	" "	*t*	1 *t*, 6		
92	" "	*t*	2 *t*, 6		
93	" "	*t*	1 *t*, 6	*t*, 23–26	
94	" "	*o*	2 *s*, 7	*s*, 15–17, 25–27	
95	" "	*o*	*o*	*s*, 23–26	
96	" "	*t*	1 *t*, 4	*t*, 22–25	
97	" "	*s*	*o*	*s*, 23–26	
98	" "	*t*	1 *t*, 4		
	Vicinity of Douglas Lake, Michigan				
	Sedge Pond.				
99	July 1919	immature	*o*	*o*	*t*, 6:*s*, 7
100	" "	"	*o*	*o*	*t*, 6,7:*s*, 8
101	" "	"	*o*		*t*, 6:*ss*, 7
102	" "	"	*o*		*t*, 6·*s*, 7
103	Aug 1919	*o*	*o*	*s*, 23–26	
104	" "	*s*	*o*	*s*, 23–26	
105	" "	*s*	*o*	*s*, {23–25; 23–26}	
106	" "	*t*	*o*	*t*	*s*, 6
107	" "	*t*	*o*	*t*, 23–26	*s*, 6
108	" "	*t*	*o*	*t*	*s*, 6
109	July 1919	*t*	*o*		*s*, 6
110	" "	*t*	*o*		*s*, 6
111	" "	*t*	*o*		*s*, 6
112	" "	*t*	*o*	*t*	*s*, 6
113	" "	*t*	*o*	*t*	*s*, 6
114	" "	*o*	*o*	*s*	*s*, 6
115	" "	*t*	*o*		*s*, 6
116	" "	*t*	*o*		*s*, 6
117	" "	*t*	*o*		*s*, 6
118	" "	*t*	*o*		*s*, 6
119	" "	*t*	*o*		*s*, 6
120	" "	*t*	*o*		*s*, 6
121	" "	*o*	*o*	*s*	*t*, 6
122	" "	*o*	*o*	*s*, 23–26	*t*, 6

TABLE IV, concluded

No. of specimen	*Date of Collection*	*Clitellum*	*Anterior glands*	*Posterior glands*	*Pharyngeal glands*
	Bessey Creek.				
123	July 1919	*t*	*o*	*s*, 23–26	*s*, 6
124	July 1920	*t*	*o*	*t*, 23–26	
125	" "	*t*	*o*		
126	Aug. 1920	*t*	*o*		
127	" "	*t*	*o*		
	Northwest Shore of Lake.				
128	Aug. 1920	*t*	*o*		*s*, 6
129	" "	*t*	*o*	*t*, 23–26	*ss*, 6
	Maple River.				
130	July 1919	*t*	*o*		*s*, 6
131	" "	*t*	*o*		*s*, 6
132	Aug. 1920	*t*	*o*		
133	" "	*t*	*o*		
134	" "	*t*	*o*	*t*, 23–26	
135	" "	*t*	*o*		
	Hook Point.				
136	Aug. 1920	*t*	*o*	*t*, 23–26	
137	" "	*t*	2 *t*, 3		
138	" "	*t*	*o*		*s*, 6
139	" "	*t*	2 *t*, 3	*t*, 23–26	*s*, 6
	Diogenes Pond.				
140	June 1920	*t*	*o*	*t*, 23–26	*s*, 6
141	" "	*t*	1 *t*, 3		*s*, 6
142	July 1920	*t*	1 *t*, 3, 4		*s*, 6
143	" "	*t*	*o*		*s*, 5:*o*, 6
144	" "	*t*	2 *t*, 3		*ss*, 6
145	" "	*t*	2 *t*, 3		*s*, 6
146	June or July 1920	*t*	*o*		
147	" " "	*t*	1 *s*, 3		
148	" " "	*t*	1 *t*, 3		
149	" " "	*t*	*o*		
150	" " "	*t*	1 *s*, 3		
	Burt Lake, Carp Creek.				
151	Aug. 1920	*t*	2 *t*, 10	*t*, {24–26, 25–26}	*s*, 6
152	" "	*t*	*o*		*ss*, 6
153	" "	*t*	*o*		
154	" "	*t*	*o*	*t*, 23–26	
	Burt Lake, Maple River.				
155	Aug. 1920	*t*	*o*	*t*, 23–26	*s*, 6

ations in shape, due to the configuration of the cœlome and possibly to the contraction of the muscles of the gland. In several worms there is a slight, and in No. 151 a very definite epidermal thickening surrounding the gland pore. Typically, the anterior glands open into the follicles of setae *a* of the somites in which the glands are located. These setae are not ornamented or otherwise different from the usual type, but they are lacking in two worms. No. 36 lacks setae *a* and has glands which are larger than usual and which have a heavier muscular layer. No. 151 lacks setae *a* and *b*. Since No. 36 is the only mature worm available from its locality, and No. 151 is the only one from its locality which was studied and which has glands, it is not known whether their conditions are exceptional or usual for their respective localities.

Variations in number and position of the anterior glands are numerous. These glands occur most frequently in somites 3, 4 and 6. They are in somite 7 of three worms; in somite 8 of two worms, and in somite 10 of one worm. Most of the specimens which have the glands in 6 are from Havana, Illinois (1920); most of those which have them in 4, from Homer Park, Illinois and the earlier collections at Havana; most of those which have them in 3, from Michigan and the earlier collections at Havana. There may be a pair of glands symmetrically placed in the somite, or there may be a gland on one side only. Each of about half of the worms from the earlier collections at Havana has three or four glands in somites 3 and 4. None of the recently collected worms have more than two glands, although one of them (No. 142) has its two glands in two successive somites.

A summary of the condition of the anterior glands in the worms collected in different localities shows that the glands are not always present. Of a total of 46 worms from Homer Park, 39 have anterior glands in somite 4, and seven have no anterior glands; of 16 worms from Havana (1920) eight have glands in 6; two, in 4; two, in 7; and four lack glands. Among the Michigan specimens, the 22 from Sedge Pond have no anterior glands; the same is true of five from Bessy Creek, two from the northwest shore of the Lake, and six from Maple River. Of four worms from Hook Point, two have glands in somite 3, and two lack glands; of eleven from Diogenes Pond, seven have glands in 3, and four lack glands. Three worms from Carp Creek lack glands and one has glands in 10. Of eleven worms from the earlier collection at Havana, seven have glands in somites 3 and 4; one, in 4; one, in 6; one, in 7; and one lacks glands. Of nine worms previously collected at Douglas Lake, one has an anterior gland in 4, and all the others have glands in 3.

In all these groups mentioned, only certain ones from the Douglas Lake region have a uniform condition in respect to the anterior glands. These are the Sedge Pond, Bessey Creek, and Maple River groups, in all of which such glands are lacking. It is possible that a study of more speci-

mens from Bessey Creek and Maple River would result in finding some worms with glands. Of the groups of four or more worms which were collected in 1919 and 1920, and in some of which glands are present, the percentage having glands varies from 25 to 85. The significance of these percentages lies in the fact that they show a wide variation. The position of the glands is least variable in the worms from Homer Park. All of these have the glands in somite 4. The worms with the greatest variation in the position of the glands are those from Havana. Those of the 1920 collection have glands in somites 4, 6 and 7, with most of them in 6; those of the earlier collections have glands in somites 3, 4, 6 and 7, with most of them in 3 and 4.

The fact that glands, probably similar in function and differing only slightly in structure, are found ventrally in different worms in several of somites 3, 4, 6, 7, 8, 10, 15, 16, 17, 22, 23, 24, 25, 26 and 27 suggests that these glands are remnants of a once continuous series of ventral glands. Eisen (1896) wrote: "Of the same nature (as the spermiducal glands) I consider the forward parietal glands in somite 3 of *S. eiseni*, and it seems not unlikely that originally this genus possessed many more pairs of spermiducal glands, perhaps one in every somite." In order to account for the two kinds of accessory glands, it would have to be assumed that there had been two series of glands, or that one series had become differentiated into two kinds of glands. The facts that all of the earlier (1913) collection of worms from Douglas Lake, probably Diogenes Pond, have anterior glands, but that over 33 1/3% of those of the 1920 collection from Diogenes Pond, which were studied, lack these glands; and that over half of the earlier (1895) collection of worms from Havana had three or four glands, while none of those from the recent collections have more than two glands, suggest the possibility that these changes may be continuing even now. Because of the variable condition of these glands, it scarcely seems that their presence or absence can be regarded as a specific character.

The first mention in the literature of anything that seems comparable to the accessory glands described above, is the description (Perrier 1874) of 40 pairs of pyriform bodies located in the posterior somites of *Pontoscolex* (originally *Urochaeta*) *corethrurus*. Dissection and sections of a few somites of a specimen of *P. corethrurus*, which is in the collection of Professor Smith, have established the fact that each pyriform body is part of a nephridium.

In the glossoscolecid genera, Andiorrhinus, Criodrilus, Kynotus, Microchaeta, Rhinodrilus and Tritogenia, glands have been described which are associated with the ventral setae. In Criodrilus these are conspicuous glandular areas about each pair of ventral setae on the clitellum. Some of the setae are modified as genital setae. In the genus Microchaeta, the glands are located in some one or all of somites 9 to 35. There may be a

single gland or there may be several glands opening into a setal follicle. Some of the setae are modified as genital setae, and sometimes external papillae mark the location of the glands. The glands of *M. papillata* (Benham 1892) and of a specimen of *M. algoensis* in the collection of Professor Smith, are structurally like those of *Sparganophilus eiseni*, except that those of *Microchaeta algoensis* have both circular and longitudinal bands in the muscular layer.

Such structures are not unknown among Lumbricidae. The so-called papillae, on which setae *ab* of 9, 10 and 11 of *Helodrilus caliginosus trapezoides* are located, are thickenings of the epidermis into which irregular projections of the cavity of the setal follicle extend. There are glandular structures accompanying the copulatory setae of *Lumbricus terrestris* (Hering), and the ventral setae of 13-16 of *Bimastus palustris* (H. F. Moore).

Glands are associated with ventral setae, which in some are modified as genital setae, in the megascolecid genera, Acanthodrilus, Octochaetus, Dichogaster, Diplocardia and Pheretima. Diplocardia (Eisen 1900) has glandular areas consisting of cells which lie between the epidermal and muscular layers and which have long ducts opening into the setal follicles. In other genera the relation of the glands near the genital and spermathecal orifices to the ventral setae is not known, but from brief accounts of their structure it seems probable that they are similar to the accessory reproductive glands described. The accessory glands described by Sweet are not connected with the setae but with the sperm duct.

In one of the Moniligastridae, *Syngenodrilus lamuensis* (Smith and Green), "prostates" have been described in somites 11-13. They are not connected with the sperm ducts which open on the anterior margin of 13. Each gland opens to the exterior slightly laterad of seta *b*, and has a tubular lumen surrounded by epithelial, glandular and muscular layers. Beddard (1887) described a similar position for the muscular layer in the prostates of *Eudrilus eugeniae* (originally *sylvicola*), but in all other glands of the accessory type of which descriptions have been found, the muscular layer is between the epithelial and glandular layers.

The glandular structures which have just been mentioned are so variable and the data on their development and structure are so meager that it is difficult to interpret their relations to each other. Lankester suggested that the capsulogenous glands of Lumbricus were excessive developments of the setigerous glands. Beddard (1895) concluded that the accessory, or copulatory glands as he called them, had developed as a result of the invagination of a glandular area, and that the accessory and prostate glands were serially homologous. It was suggested, also by Beddard, that the glands may have developed differently in different families of earthworms. Stephenson and Haru concluded that the prostate glands of *Pheretima hawayana* were mesodermal in origin, whereas it had

previously been assumed that all prostates and accessory glands were ectodermal. It has been shown, (p. 21) that the glands of *Sparganophilus eiseni* are ectodermal in origin.

The accessory glands have been thought by different investigators to produce slime, egg capsule (cocoon), albumen, a poisonous secretion, or an irritating secretion. Rosa thought that the glands of *Microchaeta benhami* had assumed a prostate function. Eisen (1896) held a similar view in regard to the glands of *Sparganophilus eiseni*. Benham (1892) stated that the copulating individuals of Lumbricus were held together by a sucking action of the clitellum and not by a slime tube. He, accordingly, attributed a sucking action to the accessory glands about the setae of *Microchaeta papillata*. Buchanan suggested that the secretion of the accessory glands which are near the spermiducal and oviducal pores of *Notoscolex* (originally *Cryptodrilus*) *saccarius* might aid in the passage of the body of the worm through the cocoon without friction. Since these last mentioned glands are not related to the setae, they may not belong to the same category as those under discussion, and yet they seem to be similar to those of Pheretima which Beddard (1895) considered capsulogenous.

No evidence for a definite conclusion in regard to the function of these glands has been obtained from the present study. The writer did not see any living specimens of *Sparganophilus eiseni in copulo*, but did study sections of a pair of worms *in copulo* which had been collected by Professor Smith at Havana, Illinois in 1895. As is generally the case in copulating earthworms, the anterior ends are turned in opposite directions and are closely approximated along their ventral surfaces. The ventral side of somites 18-27 is concave and somites 1-9 of the opposite worm lie in this concavity. These parts of the two worms are held closely together by a slime tube which is constricted at both ends. There is such a slime tube about one anterior end of each of two copulating pairs, but the other anterior ends have evidently pulled away from the opposed somites. It is probable that the slime tube was continuous from somites 1-27 of both worms.

The anterior end of the worm is pushed into the concavity of somites 18-27 so far that setae *cd* of somites 1-9 are just outside the ventro-lateral edges of somites 18-27. Setae *cd* of somites 26 and 27 are on the convex surface of those somites but close to their ventro-lateral edges. The closest contact of the worms is in and immediately below the seta line, *c*, of somites 1-9. In sagittal sections the epidermal cells of the two worms are so intimately associated in places that the dividing line between them is not readily recognizable. Medially there is an irregular slit-like space between the worms.

The slime tube apparently is not formed as a single tube enclosing the worms, but by the fusion of two tubes, one around each worm. A thickening in the slime tube, just laterad of the line of contact of the worms, is

interpreted as the line of fusion of the two tubes. While the slime, which forms the tube enclosing the worms, is thin and deeply stained, that which is between the worms is thicker and only faintly stained. Dorsally the slime tube does not extend deep into all the narrow crevices of the intersegmental grooves, and does form a smooth, continuous outer surface, which obliterates the outlines of segments. Ventrally the slime covering extends into the various grooves, although not always closely, and forms a small mass filling the anterior end of the slit-like space between the worms. The cuticula is recognizable in different places, both dorsally and ventrally, between the slime and the epidermis.

The accessory reproductive glands of somites 7, 25, 26 and 27 open into the irregular slit-like space between the worms. The pores of the accessory glands are not in contact with the body of the opposed worm, therefore, these glands cannot be sucking organs as was suggested for similar structures in *Microchaeta papillata*. The spermathecal pores open very close to, but slightly laterad of the line of closest contact. The spermiducal pore could not be found. The intersegmental grooves 19/20 and 8/9 are opposite each other. Since the accessory glands open into the median space, from which the spermathecal pores are apparently separated, it hardly seems that the secretion of the former can facilitate the transfer of spermatozoa, if this transfer is accomplished as it is in Lumbricus (Andrews).

Different investigators have thought that these glands helped in the formation of the slime tube which is present on worms *in copulo* and at the time of cocoon formation. Since the posterior glands are in somites at the posterior end of the clitellum, and the anterior glands in somites opposite the posterior end of the clitellum of worms *in copulo*, and since these glands open on the ventral side where the clitellum is thin, it is possible that they do produce a part of the slime tube, or the slime which plugs up the opening at the anterior end of the worm. The glands of 15, 16 and 17, when present, are between the clitella of the copulating worms and might help in the completion of the slime tube. Or, the secretion of the accessory glands might help to fasten the slime tubes together.

This study of the accessory reproductive glands shows that the presence and position, especially of the anterior glands, is variable; and that the function of both anterior and posterior glands is related to the reproductive activities but is not definitely known.

VI. Systematic Relations

Eisen (1896) first reported the presence of the anterior accessory reproductive glands in *Sparganophilus eiseni*. Since he did not find such glands in other specimens of Sparganophilus from Central and North America, he concluded that these glands distinguished *S. eiseni* from all other species. Because of the presence of such glands and of some minor

differences, he separated *S. eiseni* from *S. benhami* and the less distinct species, *S. guatemalensis* and *S. carneus*. He also stated that he had insufficient well-preserved material of the two latter species, and that both might prove to be varieties of *S. benhami*.

The most important difference between *S. benhami* and *S. eiseni*, as defined by Eisen, was the presence in the latter of the anterior accessory reproductive (parietal) glands. In the present discussion of these glands it has been concluded (p. 29) that they are too variable in presence and absence to be properly regarded as a basis for separating species. These species were also said to differ in size; in position of the tubercula pubertatis, and of the anterior nephridia; in the shape of the spermathecae; in the lobulation of the sperm sacs and in the relative sizes of the pharyngeal (septal) glands. Again facts have been presented to show that there is quite a variation in each of these characters among the specimens of *S. eiseni* studied, and that there are conditions among some of these specimens which are similar to the conditions described in *S. benhami*. A specimen, identified by Eisen as *S. benhami*, is in the collection of Professor Smith. It does not have the clitellum limited ventrally to somites 17-26, as described for the species, but on 15-27, with a thicker portion on 22-27. It also has the spermiducal pores on somite 19, not on 20. It has no dorsal pores. Sections reveal nothing which is different from conditions found in *S. eiseni*. Therefore it seems that *S. benhami* should be united with *S. eiseni*.

Since the presence of anterior glands is insufficient to distinguish species, the only significant difference between *S. guatemalensis* and *S. eiseni* is the fact that the clitellum in the former is on somites 16-26. In a specimen of *S. eiseni* collected in October, the clitellum appears to begin on somite 16, probably because the clitellum was degenerating. Since the material, on which the description of *S. guatemalensis* was based, was not in good condition; since the difference is small, and especially since, in other points, it agrees with the conditions found in *S. eiseni*, there seems to be insufficient basis for making this a separate species. The differences between *S. carneus* and *S. eiseni* are also such as may be accounted for in the variations of the latter. Eisen suggested that *S. carneus* might be a northern form of *S. benhami*, and mentioned that the former resembled *S. eiseni* in the shape of the spermathecae.

In the original description of the genus Sparganophilus, Benham placed it in the family Rhinodrilidae, which he had defined (1890), but which has since been made a part of the family Glossoscolecidae. Michaelsen (1917) united the Glossoscolecidae and the Lumbricidae into the Lumbricidae *s. l.*, and subdivided the former Glossoscolecidae into five subfamilies: Glossoscolecinae, Sparganophilinae, Microchaetinae, Criodrilinae and Hormogastrinae. These were given equal rank with the Lumbricinae, formerly Lumbricidae, in the new family Lumbricidae *s. l.* This was done

because additional studies had made a separation of the Glossoscolecidae and Lumbricidae, as two families, seem to him impracticable. According to this classification, Sparganophilus in the only genus of the subfamily Sparganophilinae. Whereas, it was formerly considered the ancestral form of the Glossoscolecidae, Michaelsen now considers it a degenerate descendant of the Glossoscolecinae. In a more recent paper (1921), Michaelsen has created a Familienreihe Lumbricina, in which he has placed the families Glossoscolecidae, Sparganophilidae, Microchaetidae, Hormogastridae, Criodrilidae and Lumbricidae.

VII. Summary

1. The embryology, as far as it was followed, presents no marked differences from that of other earthworms.

2. Aside from the somewhat greater histological differentiation, the nephridium of *Sparganophilus eiseni* is similar to that part of the nephridium of Lumbricus, which extends from the nephrostome to the muscular duct. There is no muscular duct in the nephridium of *Sparganophilus eiseni*.

3. The nephridia begin to develop in somite 3 and the somites posterior thereto in embryos of *S. eiseni*. Disintegration soon sets in at the anterior end, and causes the complete loss of the nephridia of somites 3-11 and the loss or degeneration of the nephridia of 12 and 14, and sometimes of 13.

4. The genital ducts and funnels, although closely associated with the nephridia, develop independently of them.

5. The accessory reproductive glands, of the various specimens examined, are found in three or more of 15 different somites, and probably are remnants of a once continuous series of glands. The anterior accessory reproductive glands are too variable to be of value in distinguishing species.

6. *Sparganophilus eiseni* is a variable species in several respects. Since each of the combinations of structural characters which are included in the descriptions of the various species: *S. benhami*, *S. guatemalensis* and *S. carneus* are found represented among various individuals of *S. eiseni*, it seems necessary to unite them into the one species, *S. eiseni*.

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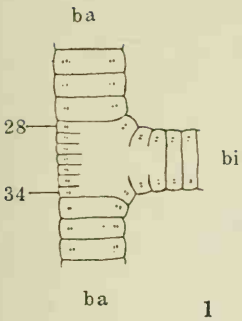
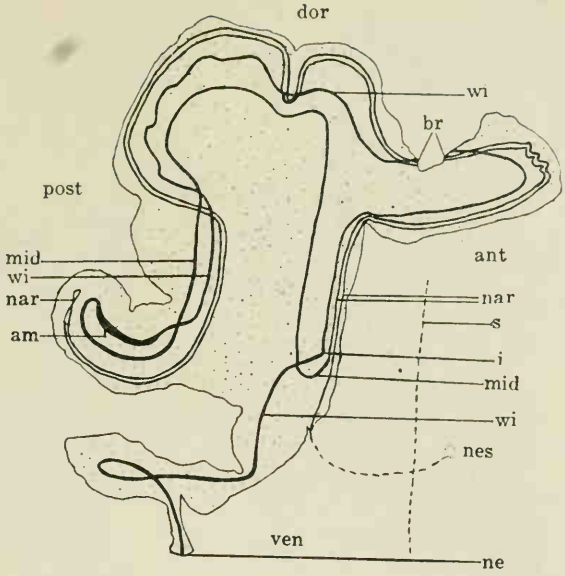
EXPLANATION OF PLATES

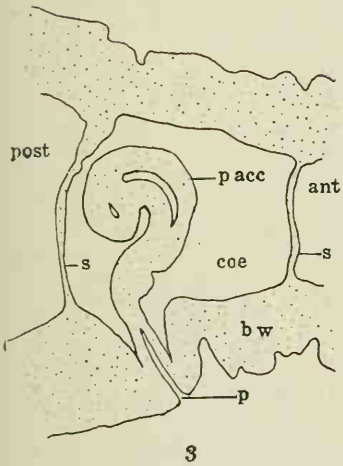
ABBREVIATIONS

a acc.	anterior accessory reproductive gland
am.	ampulla
ant.	anterior
b a.	body axis
bi.	branch
br.	break in nephridium
bv.	blood vessel
b w.	body wall
bww.	width of body wall
c.	cuticula
ca.	canal
ce.	nephridial cell
cir.	connection with circular muscles
cm.	circular muscles
coe.	cœlome
con.	constriction between middle and wide tubes
deg.	mass of degenerate muscular and glandular tissues
dor.	dorsal
d sac.	dorsal sac
e.	cœlomic epithelium
en.	nucleus of epithelial cell
ep.	epidermis
epn.	nucleus of epidermal cell
gl.	glandular cells
gl e.	glandular epithelium
gr.	granular layer
i.	beginning of last part of wide tube
i cy.	inner cytoplasm
lc.	large cell
lm.	longitudinal muscles
lu.	lumen
mi.	mitotic division
mid.	middle tube
mu.	muscular layer
n.	nucleus of nephridial cell
na.	cut edge of narrow tube
nar.	narrow tube
n c.	nerve cord
ne.	nephridiopore
nes.	nephrostome
n lc.	nucleus of large cell
p.	gland pore
p acc.	posterior accessory reproductive gland
p cy.	peripheral cytoplasm
phar.	pharynx
post.	posterior
reg.	regenerating epithelium
s.	septum
se.	seta
se f.	setal follicle
se m.	setal muscles
th w.	thin walled part of wide tube
tk w.	thick walled part of wide tube
ven.	ventral
wa.	waste in ampulla
wi.	wide tube
28, *34*,	somite numbers

All drawings except figures 1 and 2 were made with the aid of a camera lucida.







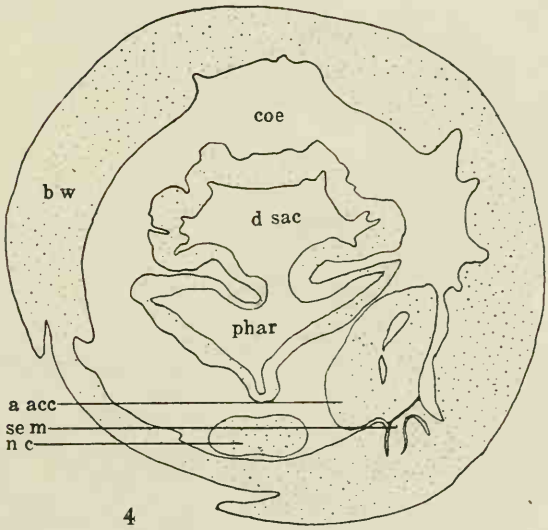


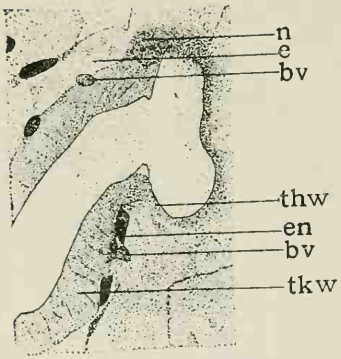
PLATE I

Fig. 1. Outline of the bifurcation in a young worm. Dorsal view.

Fig. 2. Outline of a nephridium and its canal.

Fig. 3. A somite with a posterior accessory reproductive gland. Longitudinal section. X 45.

Fig. 4. Somite 4 with an anterior accessory reproductive gland. Cross section. X 45.



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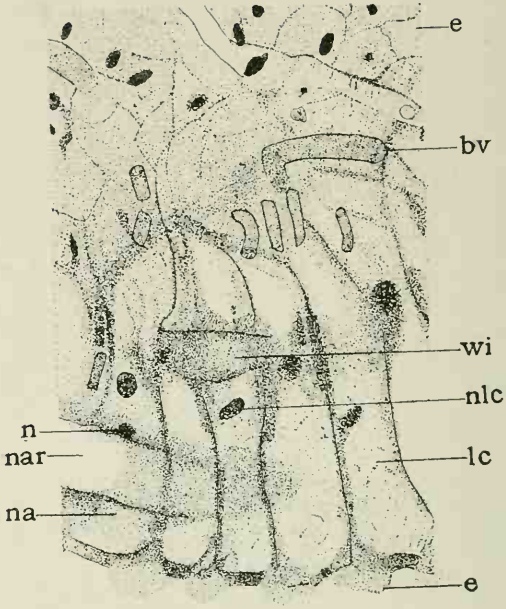

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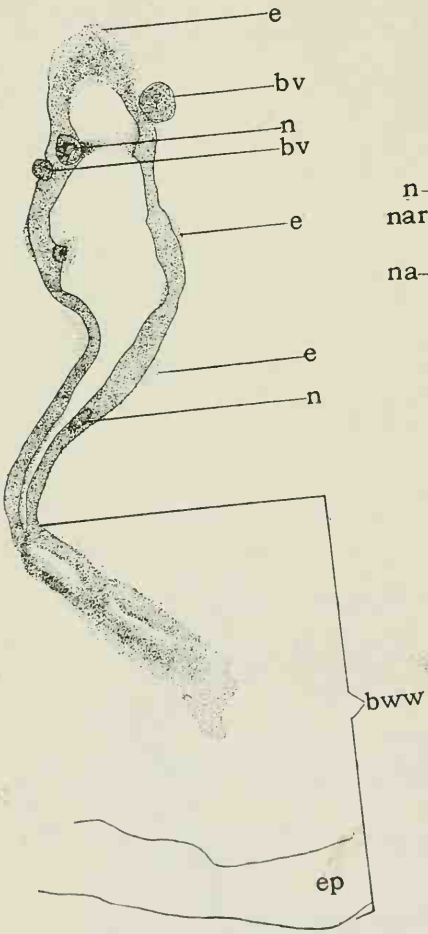

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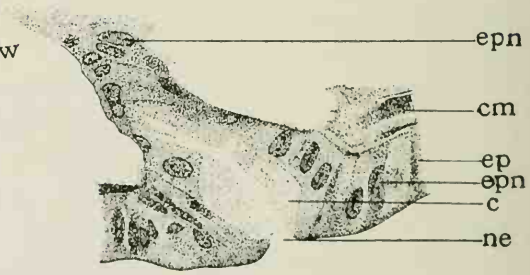
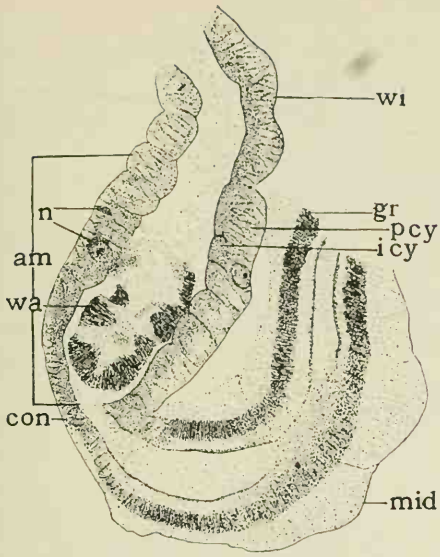


8

PLATE II

Fig. 5. Section through the wide tube at *i* (fig. 2). X 469.
Fig. 6. Part of a section through a nephridium. X 469.
Fig. 7. Section through the wide tube at its entrance into the body wall. X 469.
Fig. 8. Section through the nephridiopore. X 469.



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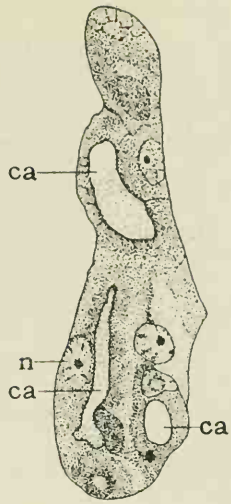

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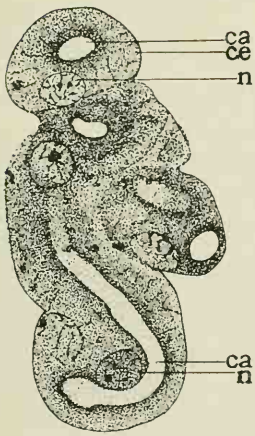

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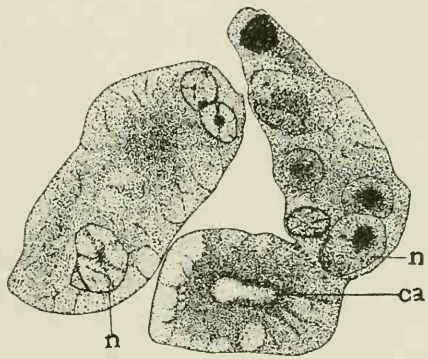

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PLATE III

Fig. 9. Section through the junction of middle and wide tubes. X 469.

Fig. 10. Section through a developing nephridium. X 1173.

Fig. 11. Section through a developing nephridium of a 0.5 mm. embryo: an earlier stage than figure 10. X 1173.

Fig. 12. Section through a typical young nephridium. X 1173.

Fig. 13. Part of a section through a disintegrating nephridium. X 1173.

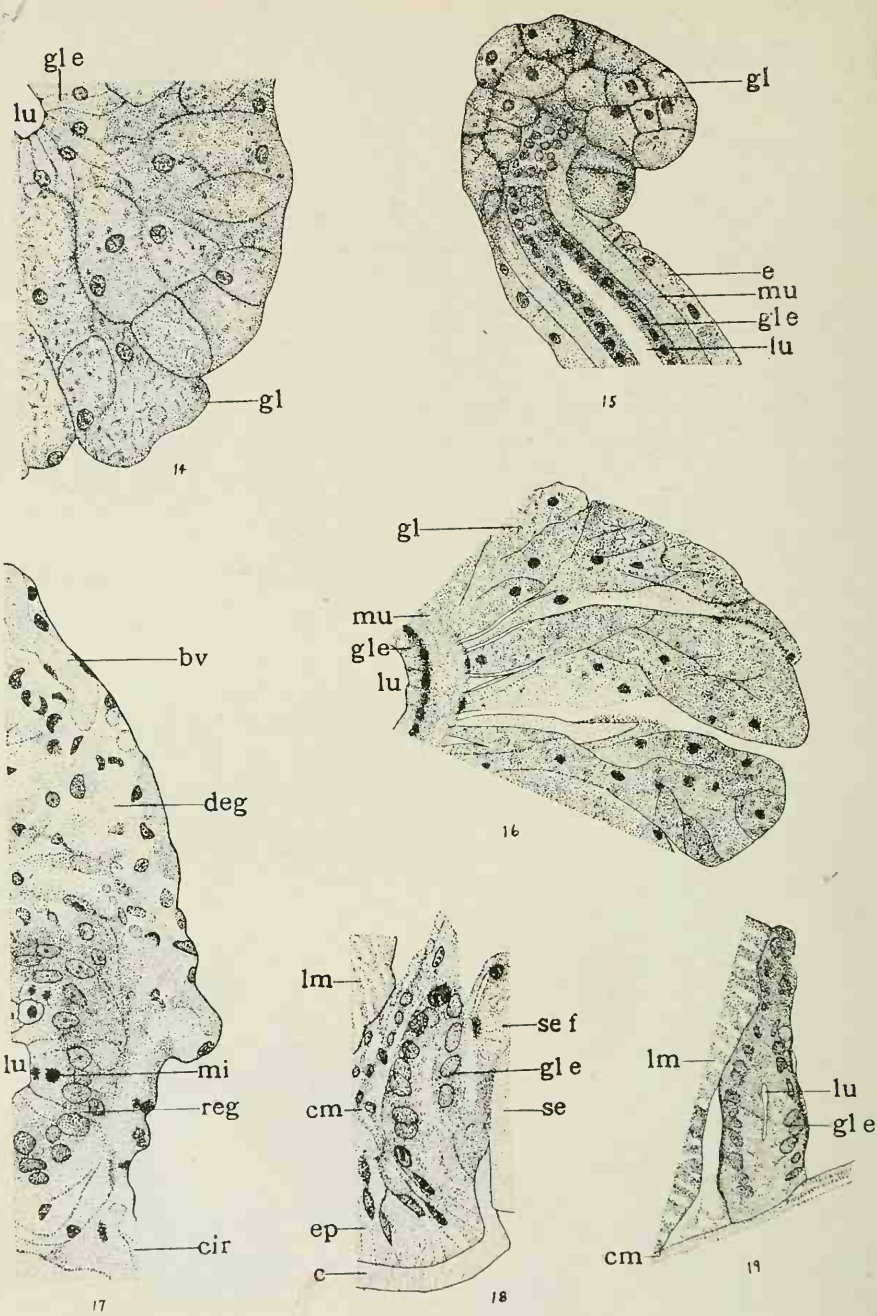

PLATE IV

Fig. 14. Posterior accessory reproductive gland; part of a section through the glandular portion. X 300.

Fig. 15. Posterior accessory reproductive gland; section through the transition of duct into glandular portion. X 300.

Fig. 16. Anterior accessory reproductive gland; part of a section through the glandular portion. X 300.

Fig. 17. Part of a section through a degenerate anterior accessory reproductive gland. X 514.

Figs. 18 and 19. Sections through a developing anterior accessory reproductive gland.

Fig. 18. The invagination. X 514.

Fig. 19. The inner part. X 514.

A SYSTEMATIC PRESENTATION OF NEW GENERA OF FUNGI

By

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The new families and genera of fungi described since volume 22 of Saccardo's "Sylloge Fungorum" was compiled are here assembled from all available literature and presented in a concise, classified form with the reference accompanying each new name. This paper is necessarily incomplete because some publications and parts of others were unavailable. Only small parts of Broteria, The Botanical Magazine of Tokyo, and Österreichische Botanische Zeitschrift were found.

As far as is known, there has been no previous compilation of the new genera of fungi described since 1910. Mycologists have been compelled to search through an extensive, scattered literature for the special types in which they were interested. This paper will be of value to mycologists in that it contains, with necessary references, most of the new genera of fungi published since the last volume of the "Sylloge Fungorum" was compiled.

The authors wish to thank Dr. F. L. Stevens for numerous helpful suggestions. To him is due the credit for planning the work of assembling all the new families, genera, and species of fungi described since 1910. They also wish to acknowledge the assistance of Messrs H. L. Dixon, J. M. Mendoza, P. J. Byrd, H. C. Abbott, and Miss Ruth Dowell who aided in the search through the literature.

Approximately 7,000 new species of fungi were listed on cards and catalogued in taxonomic order. The cards bear the citation, classification, the name of the genus, species, and generally the host of the fungus. Of these about 800 belong in the Sphaerioidaceae, 700 in the Agaricaceae, 300 in the Pucciniaceae, 200 in the Dematiaceae, 200 in the Microthyriaceae, 200 in the Pleosporaceae, 150 in the Mycosphaerellaceae, and 100 in each of the following families: Dothideaceae, Hypocreaceae, Melanconiaceae, Moniliaceae, Polyporaceae, Sphaericaeae, Thelephoraceae, Tubercularaceae, and Valsaceae. This list is too voluminous to print at the present time.

Explanation of Tables

The name of the new genus is given in the first column. The name in the second column is that of a genus nearly related or similar to the new

genus named in column 1 or sometimes some other significant name. A number has been given to each publication. This number is given in the 3rd column and refers to the bibliography. The number in column 4 refers to the volume, that in column 5 to the page, and the one in column 6 to the date of publication.

At the end of the list is a group of new genera the positions of which were not definitely stated by the authors and the affinities of which were not sufficiently clear to warrant us in assuming their relationships.

The classification system used in this paper is mainly that of Engler and Prantl as given in "Die Naturlichen Pflanzenfamilien" except in the Dothideales and Hemisphaeriales in which the keys of Theissen and Sydow are followed as closely as possible.

For the most part, the genera are classified where the authors placed them even though the positions of many were subsequently changed. See the "Synoptische Tafeln" of F. Theissen and H. Sydow in Annales Mycologici 15:389-491. 1917.

MYXOMYCETES

Plasmodiophorales

Plasmodiophoraceae

Anisomyxa	23	1		1913
Clathrosorus	9	34	467	1920
Molliardia	7	9	236	1911
Ostenfeldiella	9	28	643	1914
Sorodiscus	11	12	9, 23	1913
Sorolpidium	23		19	1911
Trematophlyctis	54	34	86	1918

PHYCOMYCETES

Chytridiales

Synchytriaceae

Mitochytridium		54	27	202	1911
Monochytrium	Synchytrium	45	10	3, 50	1910

Saprolegniales

Saprolegniaceae

Isoachlya	Achlya	3	8	231	1921
Jaraia		23		1	1913
Pythiomorpha		68	29	391	1909
Rheosporangium		82	4	280	1915

Leptomitaceae

Allomyces	Blastocksia	9	25	1023	1911

PERONOSPORALES

Peronosporaceae

Bremiella	Bremia	41	6	195	1914
Nozemia		52	13	566	
Stigeosporium	Phytophthora	9	30	357	1916

MUCORALES

Mucoraceae

Blaskestea	Choanophora	18	58	353	1914
Dissophora	Mortierella	18	58	361	1914
Haplosporangium	Mortierella	18	58	363	1914

PHYCOMYCETES STERILIA

Zoophagus		46	61	368	1911

ASCOMYCETES

PROTOMYCETALES

Hemiascaceae

Dipodascus		85	5	1	1921
Taphridium		85	5	1	1921

SACCHAROMYCETALES

Endogonaceae

Protascus		42	3	155	1913

Saccharomycetaceae

Aleurodomyces		13	26	100	1912
Blastocystis	Dermatocystis	28	71	296	
Coccidiascus		28	75	117	1913
Cycadomyces		73	3	1	1910
Guillermondia		65	23		1912
Medusomyces	Mycoderma	16	31	243	1913
Nectaromyces		22	11	176	1919
Psillidomyces		13	26	96	1912

HELVELLALES

Helvellaceae

Geomorum		89		23	1921

PEZIZALES

Pezizaceae

Aleurina	Aleuria	41	6	277	1914
Aparyphysaria		89		25	1921
Manilaea		7	12	569	1914
Mollisina		16	37	109	1919
Pezizellaster	Pezizella	7	15	349	1917

Helotiaceae

Belonioscyphella	Belonium	53	127	590	1918
Calycellina	Helotium	53	127	601	1918
Helotiopsis	Mollisiella	53	119	623	1910
Lachnobelonium	Belonium	16	37	109	1919
Lambertella	Belonioscypha	53	127	375	1918
Leptobelonium	Belonidium	16	37	108	1919
Microscypha	Dasyscypha	7	17	38	1919
Neobulgaria	Ombrophila	7	19	44	1921
Psilachnum		16	37	109	1919
Stereolachnea	Lachnea	7	15	353	1917
Tanglella		53	127	606	1919
Torrendiella	Dasyscypha	54	27	133	1911

Patelliariaceae

Pleoscutula	Scutula	54	29	434	1913
Rodwaya	Woodiella	86	13	425	1917
Siscocera	Nesolechia	59	6	48	1917

Cenangiaceae
(Including Bulgariaceae)

Asterocalyx		53	121	402	1912
Bulgariastrum		47	8	497	1913
Caloriopsis		7	15	254	1917
Caloriella	Cenangina	53	127	345	1918
Discomycella	Ascosorus	53	121	401	1912
Encoeliella		53	119	619	1910
Stegopeziza	Dermataceae	53	126	308	1917

PHACIDIALES

Stictidiaceae

Eupropolella		7	15	311	1917
Hysteropezizella	Stegia?	53	126	311	1917
Hysterostegiella	Sarcotrochila	53	126	313	1917
Phacidiella	Char. emend.	53	126	304	1917
Propoliopsis		36	6	2279	1914
Sarcotrochila	Trochila	53	126	310	1917

Tryblidiaceae

Odontoschizon	Heterosphaeria	7	12	568	1914

Phacidiaceae

Leptophacidium		53	127	332	1918
Myxophacidiella		53	126	303	1917
Myxophacidium		53	126	301	1917
Phacidiella		67	22	147	1912

HYSTERIALES

Hypodermataceae

Bifusella	Hypoderma	7	15	318	1917
Haplophyse		7	14	267	1916
Hypodermellina	Hypoderma	7	15	302	1917

		Hysteriaceae			
Hysterostomina	Hysterostomella	7	13	228	1915
Parmulina	Parmularia	7	12	194	1914
Periaster	Erikssonia	7	14	452	1916
Polhysterium	Hysterographium	8	23	87	1912
		TUBERALES			
		Tuberaceae			
Hydnotryopsis	Geopora	62	6	336	1916
		Agyriaceae			
Ramosiella		7	15	254	1915
		ASPERGILLALES			
		Aspergillaceae			
Acaulium		64	11	42	1912
Aspergillopsis		64	11	202	1912
Crollium		64	11	98	1912
Rhopalocystis		35	6	140	1911
		PERIOSPORALES			
		Erysiphaceae			
Chilemyces		31	27		1910
		7	15	454	1917
Leucoconis		7	15	456	1917
Schistodes	nov. nom.	7	15	456	1917
Typhulochaeta		19	29	22	1915
		Perisporaceae			
Acanthostoma		15	29	46	1912
Chaetostigme	Dimeriella	7	15	199	1917
Chaetostigmella	Phaeodimeriella	7	15	199	1917
Cleistosphaeria		7	14	74	1916
Diblastospermella	Dimeriorum	77	23	579	1918
Dichothrix		15	29	260	1912
Dimeriopsis	Dimerium	60	10	171	1917
Eosphaeria		7	15	362	1917
Euantennaria		77	23	549	1918
Eudimeriolum		8	23	36	1912
Guttularia	Orbicula	42	3	9	1913
Haraea		7	11	312	1913
Jaffuela		77	25	39	1921
Meliolina		7	12	553	1914
Nematothecium		36	5	1534	1912
Parodiella	Char. emend.	7	15	132	1917
Parodiopsis	Parodiella	55	31	22	1915
Perisporiopsis	Perisporium	60	10	170	1917
Phaeodimeriella		15	29	45	1912
Phaeostigme	Dimerium	7	15	199	1917
Phycopsis	Seuratia	27	154	1480	1912
Pilene		7	14	409	1916

Plenophysa		7	17	142	1919
Pseudoparodia	Dimerosporina	7	15	138	1917
Rizalia		7	12	546	1914
Rhizogene	Lasiobotrys	7	18	181	1920
Rhizosphaerella	Perisporium	32	59	254	1917
Setella		7	14	359	1916
Stigme	Dimerina	7	15	199	1917
Stomatogene		7	14	404	1916
Teratonema		7	15	180	1917
Trichospermella		8	23	38	1912
Winteromyces	Parodiella	8	23	37	1912
Englerulaceae		81	66	296	1916
Diathrypton		47	21	137	1922
Euthrypton		70	66	296	1916
Linotexis	Parenglerula	7	15	198	1917
Oothecium		77	23	519	1918
Ophiotexis		70	66	296	1916
Phaeoschiffnerula		20	12	21	1914
Rhizotexis		7	15	141	1917
Syntexis		70	66	296	1916
Theissenula	Schiffnerula	7	12	198	1914
Thrauste		70	66	296	1916
	Capnodiaceae				
Adelopus	nov. nom.	7	15	482	1917
Aithaloderma		7	11	257	1913
Antenella		7	15	473	1917
Balladynopsis	Balladyna	7	15	435	1917
Balladynella		7	15	478	1917
Calyptra		7	15	478	1917
Ceratochaete	Sitella	7	15	179	1917
Chaetothyrina		7	11	495	1913
Chrysomyces		7	15	475	1917
Crytopus		7	12	72	1914
Microtyle		77	23	458	1918
Neohoehnelia		7	15	476	1917
Phragmicapnis		7	15	480	1917
Schizocapnodium	Capnodium	49	6	91	1921
	Parodiellaceae				
Acantharia		7	16	15	1918
Epiphyma		70		306	1916
Hypophlegma		7	15	135	1917
	Parodiellinaceae N. Fam.	4	16	21	1918
	HEMISPHAERIALES				
	Hemisphaeriaceae				
Chaetoplaca		7	15	232	1917
Dictothyriella		20	12	92	1914
Dictyothyrium	Dictyopeltis	46	62	277	1912
Epipeltis		1	7	3, 26	1913
Eremotheca	Eremothecella	7	15	235	1917

Eremothecella	Phragmothyriella	7	15	236	1917
Haplopeltis		20	12	88	1914
Hormopeltis		8	23	84	1912
Myiocoprella		44	23	199	1916
Plochmopeltis		20	12	87	1914
Polyclypeolum	Microthyriella	7	12	67	1914
Stephanotheca		47	9	180	1914
Stomiopeltis		20	12	85	1914
Stomiopeltella		20	12	85	1914
	Trichopeltaceae N. Fam	26	39	625	1913
Pycnocarpon		1	7	30	1913
Pycnoderma		7	12	563	1914
Trichopeltina		15	32	3	1914
Trichopeltula		26	39	636	1914
	Trichosphaeriaceae				
Melanopsomella		7	17	121	1919
	Microthyriaceae				
Actinocymbe		53	120	416	1911
Actinomyxa		7	15	146	1917
Actinopelte		7	11	315	1913
Amazonia		7	11	499	1913
Asterolibertia	Dimerosporium	4	16	166	1918
Asteromyxa		7	15	419	1917
Asterostemula	Asterinella	7	14	270	1916
Asterinella		20	10	101	1912
Aulographiella		7	15	367	1917
Balansina		4	16	123	1918
Calothyriella	Calothyrium	7	15	371	1917
Calothyrium		7	10	160	1912
		20		82	1914
Caudella		7	14	90	1916
Caenothyrium		7	15	417	1917
Camposa		77	25	90	1921
Chaetothyrium		77	23	522	1918
Cirsosia		4	16	127	1918
Cirsosiella		4	16	129	1918
Clypeolella		53	119	403	1910
Clypeolina		26	34	234	1912
		7	15	419	1917
Cocconiopsis		4	16	113	1918
Dictyothyrium		46	62	277	1912
Echidnodes	Lembosia	7	15	422	1917
Echidnodella	Morenoella	7	15	422	1917
Englerulaster		53	119	454	1910
Entopeltis		53	119	420	1910
Galesula		7	15	237	1917
Halbaniella		7	14	430	1916
Halbanina	Halbania	4	16	163	1918
Hariotula	Microthyrium	27	164	890	1917
Irene	Meliola	7	15	194	1917

Lembosia	Char. emend	7	11	427	1913
Lembosina		7	11	437	1913
Lembosiopsis		7	11	435	1913
Kriegeriella		7	16	39	1918
Maublancia	Asterina	4	16	159	1918
Maurodothella		4	16	124	1918
Melanochlamys		39		438	1913
Meliolaster		61	8	123	1919
Micropeltella		7	11	405	1913
Morenoina		7	11	434	1913
Mycolangloisia	Lembosia	4	16	157	1918
Niesslella		16	36	468	1918
Parenglerula		53	119	465	1910
Patouillardina		27	164	890	1917
Peltella	Myiocopron	7	15	237	1917
Phaeopeltis		83	7	1	1919
Prillieuxina	Asternella	4	16	161	1918
Protothyrium		27	164	575	1917
Pycnoderma		7	12	563	1914
Pycnopeltis		7	14	365	1916
Questeria	Dimerosporium	4	16	186	1918
Seynesiella	Myiocopron	4	16	196	1918
Sirothyriella		53	119	451	1910
Stegothyrium		53	127	382	1918
Symphaeophyma	Microphaeophyma	8	23	97	1912
Symphaster		7	13	217	1915
Thallochaete		7	11	501	1913
Thyrosoma		7	19	307	1921
Trichasterina	Asterina	4	16	172	1918
Wardina	Asterina	4	16	165	1918
Yatesula	Stephanotheca	7	15	237	1917
	Microthyriopsidaceae N. Fam.	4	16	99	1918
Leprieurina		4	16	210	1918
Manginula		4	16	99	1918
	Trichothyriaceae	15	32	3	1914
Trichothyriella		15	32	4	1914
Trichothyriopsis		15	32	4	1914
	Polystomellaceae	7	13	158	1915
Armatella	Polyrhizon	7	13	235	1915
Asterodothis		7	10	179	1912
Chaetaspis	Parmulina	7	15	279	1917
Cyclotheca		7	12	7	1914
Dothithyrella		7	16	171	1918
Ellisiodothis		7	12	73	1914
Hysterostomina	Hysterostomella	7	13	228	1915
Inocyclus	Polycyclus	7	13	211	1915
Marchalia	Char. Emend	7	13	251	1915
Melanoplaca	Marchalia	7	15	298	1917
Monorhiza	Ulcopeltis	7	13	318	1915
Monorhizina	Monorhiza	7	13	320	1915
Pleiostomella	Ulcopeltis	7	15	221	1917

Polycyclina	Polycyclus	7	13	212	1916
Rhipidocarpon		7	10	456	1912
		7	13	197	1915
Scoleonema	Dothidastromella	7	15	410	1917
Synpeltis		7	15	221	1917
	Stigmataceae N. Fam.	7	14	426	1916
Aphysa		7	15	134	1917
Isomunkia	Coccinopeltis	7	13	261	1911
Vizella		20	12	1	1914

HYPOCREALES

Hypocreaceae

Balansiopsis	Balansia	53	119	936	1910
Borinquenia		60	10	173	1917
Bronectria		77	23	563	1918
Chromocrea		41	2	58	1910
Chromocreophis		41	2	63	1910
Cylindrocarpon	Nectria	69	3	225	1913
Dextria	Calonectria	60	10	174	1917
Dialhypocrea		77	23	475	1918
Epinectria		7	15	215	1917
Epispora		54	38	84	1922
Hyalocrea		7	15	214	1917
Hyalosphaera		60	10	172	1917
Hypocreophis		77	23	480	1918
Leptocrea		7	14	87	1916
Linearistroma		53	119	938	1910
Mastigocladium		27	152	326	1911
Nectriopsis	Nectria	7	9	323	1911
Neonectria	Nectria	7	15	52	1917
Orcadia		59	5	151	1914
Patellonectria		77	25	477	1918
Phyllocrea		7	16	38	1918
Podonectria	Ophinectria	59	3	146	1920
Sterocrea		7	15	216	1917
Trailia		59	5	151	1914
Uropolystigma	Polystigma	54	36	36	1920

Dothideales

	Plectodiscellaceae N. Fam.	42	4	232	1914
Plectodiscella		42	4	232	1914
	Myriangiaeceae	7	15	438	1917
Ascostratum		7	10	41	1912
Butleria		7	12	302	1914
Symphaeophyma		8	23	97	1912

Myxomyriangiaceae

Myxomyriangium		7	11	507	1913
		7	15	438	1917

Saccardiaceae

Byssogene		47	21	144	1922
Calopeziza		47	8	499	1913

	Dothideaceae Char. emend.	7	13	174	1915
Achorella	Systremma	7	13	340	1915
Actinodothis		47	9	175	1914
Amerodothis		7	13	295	1915
Angatia		7	12	566	1914
Auerswaldiella		7	12	278	1914
Aulacostroma		47	9	176	1914
Bagnisiopsis		7	13	290	1915
Benguetia		7	15	252	1917
Botryostroma		53	120	424	1911
Castagnella		54	30	358	1914
Catabotrys	Amerodothis	7	13	247	1915
Catacauma	Sphaerodothis	7	12	280	1914
Clypeostroma		7	12	272	1914
Coccochora	Char. emend.	53	119	432	1910
Coccochorella		53	119	431	1910
Coccodothis	Phyllachora	7	12	271	1914
Coccodothella	Coccodothis	7	13	280	1915
Coccostroma	Yoshinagella	7	12	269	1914
Cyclodothis		7	11	266	1913
Cyclotheca		7	12	71	1914
Dictyochora	Curreya	7	12	275	1914
Dictyodothis	Phragmodothis	7	13	346	1915
Diplochora		7	11	60	1913
Dothidastromella		53	119	421	1910
		7	13	229	1915
Dothideopsella		53	124	70	1915
Dothidina		7	13	302	1915
Ellisiodothis	Asterula	7	12	74	1914
Elmerococcum	Coccidella	7	13	282	1915
Englerodothis	Leveillella	7	13	285	1915
Halstedia		18	69	253	1920
Haplodothis		53	20	422	1911
Haplotheciella		53	128	615	1919
Heterodothis		47	9	170	1914
Hysterostoma		7	11	509	1913
Leptodothis		7	12	268	1914
Leveillina		7	13	286	1915
Leveillella		7	13	284	1915
Melanopsamnopsis	Dothidella	76	34		1917
Metachora		7	9	400	1911
Metameris	Achorella	7	13	342	1915
Microdothella		47	9	169	1914
Microcylella		7	12	68	1914
Microphrodothis	Ophiodothis	77	23	495	1918
Ophiodothiella	Ophiodothis	53	119	940	1910
Palawania		47	9	172	1914
Parmulina	Parmularia	7	12	194	1914
Perischizon	Dothidea	7	12	265	1914
Phacodothiopsis	Phacodothis	7	12	192	1914
Phragmodothella	Achorella	7	13	343	1915

Phragmodothis		7	12	179	1914
Phragmosperma		7	14	450	1916
Phyllachorella		7	12	489	1914
Placostroma		7	12	269	1914
Polyrhizon		7	12	281	1914
Psalidosperma		7	12	571	1914
Pseudophyllachora		77	23	556	1918
Pseudosphaerella	Montagnella	53	120	425	1911
Puiggarina		77	23	485	1918
Pyrenobotrys		7	12	182	1914
Rhabdostroma	Scirrhiella	7	14	362	1916
Schizochora		36	6	1929	1913
		7	11	265	1913
Septomazzantia	Mazzantia	7	13	193	1915
Stalagmites		7	12	189	1914
Stigmatodothis		47	9	174	1914
Stigmochora		7	12	272	1914
Trabutiella		7	12	180	1914
Trichochora	Discodothis	7	13	289	1915
Trichodothis		7	12	176	1914
Uleodothis	Auerswaldia	7	13	305	1915
Uleodothella		7	12	184	1920
Yoshinagella		53	122	39	1913
Yoshinagamyces	Yoshingaia	19	26	143	1912

Phyllachoraceae

Anisochora	Catacauma	7	13	406	1915
Apiospora	Char.-emend	7	13	419	1915
Camarotella	Trabutia	7	13	370	1915
Catacaumella	Catacauma	7	13	400	1915
Dermatodothis		7	12	280	1914
		7	13	69	1915
Dictyochorella	Dictyochora	7	13	610	1915
Endothiella	Phyllachora	7	13	582	1915
Munkidothis	Trabutia	7	13	360	1915
Omphalospora	Munkidothis	7	13	361	1915
Phaeochorella	Catacauma	7	13	405	1915
Phaeotrabutiella	Trabutiella	7	13	360	1915
Phragmocarpella	Phyllachora	7	13	601	1915
Phragmocauma	Catacauma	7	13	411	1915
Podoplaconema	Omphalospora	7	19	83	1921
Rhemiodothis	Trabutia	7	12	192	1914
		7	13	363	1915
Rhopographina	Rhopographus	7	13	429	1915
Scirrhodothis	Scirrhia	7	13	419	1915
Scirrophragma	Scirrhia	7	13	423	1915
Scolecodothis		7	12	277	1914
		7	13	412	1915
Trabutia	Char. emend	7	13	347	1915
Thyriopsis		7	13	369	1915

	Montagnellaceae			
Crotone		7	13	629	1915
Epibotrys		7	13	644	1915
Haplothecium		7	13	614	1915
Hyalocurreya		7	13	646	1915
Monopus		7	13	647	1915
Montagnellina	Montagnella	53	121	387	1912
Ophiocarpella		7	13	644	1915
Rosenscheldiella		7	13	645	1915
Scirrhiachora	Diplochorella	7	13	626	1915
Syncarpella	Montagnella	7	13	631	1915
	Pseudosphaeriaceae			
Epiphyma		70	66	296	1915
Lasiostemma		7	15	218	1917
Monascostroma		7	16	160	1918
Pseudoplea		7	16	162	1918
Pyreniella		70	66	296	1915
Scleropolella		7	16	158	1918
	SPHAERIALES			
	Chaetomiaceae			
Peristomium		27	55	178	1912
	Sordariaceae			
Fimetaria		43	3	65	1910
	Coronophoraceae			
Eucanthe	Coronophora	7	15	273	1917
Heteropera		7	14	423	1916
	Sphaeriaceae			
Apiosporina		53	119	439	1910
Asterosphaeriella	Didymosphaeria	7	11	260	1913
Bakeromyces		7	15	202	1917
Bolosphaera		7	15	201	1917
Boydia		59	6	2	1919
Coccidophthora		7	11	263	1913
Dasysphaeria		8	23	60	1912
Dimerinopsis		7	15	202	1917
Entopeltis		53	119	420	1910
Gibsonia	Spermatoria	9	23	336	1909
Griposphaeria		7	16	87	1918
Haplovalsaria	Valsaria	53	128	583	1919
Herpotrichiella	Acanthostigma	7	12	472	1914
Henningsomyces	Char. emend.	53	119	460	1910
Linobolus		7	15	204	1917
Meringosphaeria		44	25	414	1918
Nematostigma		7	11	263	1913
Nematostoma		7	12	161	1914
Neokeissleria		7	17	28	1919
Neotrotteria		74	6	45	1921

Plactogene		7	14	423	1916
Plagiostromella		53	126	372	1917
Porostigme	Dimerinopsis	7	15	202	1917
Pseudopleospora		7	17	84	1919
Wageria	Acanthostigma	41	11	8	1919
Xenothecium		53	128	589	1919
	Physosporellaceae N. Fam.	53	128	557	1919
Lejosphaerella	Physosporella	53	128	577	1919
	Ceratostomataceae				
Chaetoceratostoma		14	2	144	1912
Cyanospora		41	2	209	1910
	Cucurbitariaceae				
Cucurbidortris	Cucurbitaria	7	19	201	1921
Cucurbitariella		7	14	440	1916
Keissleriella	Otthiella	53	128	582	1919
Montagnina		53	119	417	1910
		53	121	350	1912
Rostronischkia		41	11	166	1919
	Amphisphaeriaceae				
Starbackiella		7	17	37	1919
Titanella		7	17	36	1919
	Mycosphaerellaceae				
Discosphaerina	Guignardia	53	126	353	1917
Lulworthia		59	5	259	1916
	Pleosporaceae				
Acantharia	Hypolegma	7	16	15	1918
Acanthotheciella	Ophiochaeta	53	120	450	1911
Crisserosphaeria	Ophiobolus	8	23	72	1912
Didymellina	Didymella	7	16	66	1918
Epipolaeum	Hypolyma	7	16	7	1918
Phanerococcus	Hypoplegma	7	16	9	1918
Physalosporina	Physalospora	7	9	288	1911
Plectosphaeria	Physalospora	7	14	413	1916
Scleropliella	Leptosphaeria	7	16	158	1918
	Massariaceae				
Leptomassaria	Phorcys	7	12	474	1914
Myelosperma	Pseudomassaria	7	13	38	1915
Trematosphaeria	Massaria	53	123	99	1914
	Gnomoniaceae				
Desmotascus	Phomatospora	18	68	476	1919
	Stegasphaeriaceae N. Fam.	7	14	364	1916
Stegasphaeria		7	14	362	1916
	Clypeosphaeriaceae				
Amerostegi		70	66	297	1915
Euacanthe	Clypeosphaeria	7	15	272	1917
Linocarpon		7	15	210	1917
Schizostege		7	14	415	1916

Stigastroma		7	14	81	1916
Teratosphaeria		7	10	39	1912
Trabutiella	Trabutia	18	70	401	1920
	Valsaceae				
Allanthoporthe	Diaporthe	32	62	289	1920
Clypeoporthe	Diaporthe	53	128	584	1919
Discoidaporthe	Allantoporthe	32	62	293	1920
Macrodiaporthe	Diaporthe	7	17	94	1919
Phaeodiaporthe	Diaporthe	7	17	99	1919
Valseutypella	Valsa	7	18	72	1920
	Melanconidiaceae				
Amphicytostroma	Crytosporella	7	19	63	1921
Cryptoceuthospora	Crytosporella	7	19	56	1921
	Diatrypaceae				
Apioporthe	Diatrype	53	126	381	1917
Ectosphaeria	Diatrype	77	25	48	1921
Phaeotrype	Diatrype	41	12	201	1920
	Melogrammataceae				
Anisomyces		7	12	270	1914
Causalis		7	16	184	1918
Pseudothis		7	12	274	1914
Phaeobotryon	Botryosphaeria	7	13	664	1915
	Xylariaceae				
Theissenia		54	30	52	1914
	LABOULBENIALES				
	Laboulbeniaceae				
Amphoropsis	Platysthethus	10	85	312	1917
Aposporella		18	69	11	1920
Autophagomyces		48	48	172	1913
Cantharosphaeria		18	69	3	1920
Cochliomyces		8	23	180	1912
Coreomycetopsis		18	69	13	1920
Crytandromyces		48	48	173	1913
Cucujomyces	Monicomyces	8	29	506	1917
Diandromyces		48	55	209	1920
Eudimeromyces		48	55	215	1920
Endosporella		18	69	16	1920
Entomocosma	Anformorfidea	10	85	315	1917
Helodiomyces		54	29	557	1913
Ilytheomyces		48	52	704	1917
Laboulbeniella		8	23	188	1913
Laboulbeniopsis		18	69	17	1920
Mimeromyces	Sphaleromyces	48	48	163	1913
Myriapodophila	Herpomyces	10	85	313	1917
Nycteromyces		48	52	653	1917
Pselaphidomyces	Stichomyces	8	29	662	1917
Scaphidiomyces		48	48	219	1913

Scelophoromyces		48	48	210	1913
Stephanomyces	Monicomyces	8	29	671	1917
Synandromyces		48	48	174	1913
Synaptomyces		48	48	218	1913
Tengandromyces		48	48	177	1913
Termitaria		18	69	8	1920
Tetrandromyces		48	48	168	1913
Thaxteriola		10	85	314	1917
Trenomyces	Dimeromyces	54	25	155	1909

BASIDIOMYCETES

Ustilaginales

Ustilaginaceae

Anthracocystis	Ustilago	63	15	53	1912
Mycocoscoma		63	15	50	1912

Uredinales

Melampsoraceae

Botryorhiza	Endophyllum	3	4	47	1917
Crossopsora		7	16	243	1918
Endophylloides	Endophyllum	3	4	50	1917
Oliveo		41	9	61	1917

Pucciniaceae

Alevomyces	Uromyces	5	28	190	1914
Anthomycetella		7	14	353	1916
Calidion		7	16	42	1918
Caronotelium		7	19	174	1921
Cephalotelium		7	19	165	1921
Chrysocelis		39	5	542	
Clenoderma		4	17	103	1919
Cleptomyces	Calliospora	18	65	464	1919
Ctenoderma		7	17	102	1919
Cystopsora		7	8	448	1910
Cystotelium	Longia	7	19	165	1921
Desmella		7	16	241	1918
Dichlamys		7	19	105	1919
Graveola		7	19	173	1921
Gymnotelium		7	19	170	1921
Haplaravenelia		7	19	165	1921
Haplopyxis		7	17	105	1919
Kunkelia		18	63	504	1917
Linkiella		7	19	173	1921
Longia		7	19	165	1921
Miyagia		7	11	107	1913
Nielsenia		7	19	171	1921
Nothoravenelia	Ravenelia	7	8	310	1910
Nyssopsorella		7	19	169	1921
Ontotelium		7	19	174	1921
Oplophora		7	19	170	1921

Peristemma		7	19	175	1921
Phragmotelium		7	19	167	1921
Pleomeris	Nielsenia	7	19	171	1921
Sclerotelium		7	19	172	1921
Teloconia		7	19	168	1921
Trachizsporella		7	19	168	1921
Triactella		7	19	169	1921
Tricella	Calliospora	41	4	283	1912
Trocochodium		7	17	106	1919
Triphragmiopsis		54	30	78	1914
	Uredinales Imperfecti				
Argomyces		43	7	216	1912
Xenostele		7	18	178	1920
	Auriculariales				
	Auriculariaceae				
Hoehnelomyces	Pilacrella	16	37	514	1919
	Tremellales				
	Tremellaceae				
Gloeosoma		7	18	51	1920
Phaeotremella		59	5	376	1911
	Dacryomycetales				
	Dacryomycetaceae				
Dacryopsella		53	124	49	1915
	Agaricales				
	Thelephoraceae				
Duportella	Stereum	47	10	87	1915
Jaapia		7	9	428	1911
Peniophorina	Peniophora	53	126	285	1917
	Hydnaceae				
Gloiothele		7	18	44	1920
Hydnodon	Hydnum	41	5	297	1913
	Polyporaceae				
Echidnodia		54	34	199	1918
Ermeria	Daedalea	32	51	318	1912
Pseudopolyporus		41	2	93	1910
Xanthoporia		41	8	56	1916
	Agaricaceae				
Amanitella	Amanita	7	11	337	1913
Catathelasma		18	50	383	1910
Chlorophyllum		43	9	172	1910
Chlorosperma		41	14	96	1922
Copelandia		32	53	51	1913
Lentodiellum		41	7	216	1915
Micropsalliota	Agaricus	53	123	79	1914

Plicaturella		43	9	172	1910
Polyozellus		33	9	171	1910
Rhodopaxillus	Tricholoma	7	11	337	1913
	PHALLALES				
	Phallaceae				
Protophallus	Phallogaster	41	2	25	1910
	Clathraceae				
Pharus	Lysurus	83	7	60	1919
	HYMENOGASTRALES				
	Hysterangiaceae				
Jaczerwskia		37	63	214	1913
Phaeocryptopus	Cryptopus	54	30	424	1914
	Hymenogastraceae				
Stephanospora	Hydnangium	54	30	349	1914
	Scleroderma taceae				
Neosaccardia	Scleroderma	75	56	6	1921
	LYCOPERDALES				
	Lycoperdaceae				
Geasteroides	Geasteropsis	41	9	271	1917
Lycoperdellon	Lycoperdon	20	11	92	1912
	FUNGI IMPERFECTI				
	SPHAEROPSIDALES				
	Sphaerioidaceae				
Amphiciliella		32	62	58	1920
Amphorula	Kellermania	43	60	82	1922
Amylirosa	Ephelidium	10	90	178	1920
Angiopomopsis		53	121	407	1912
Bakerophoma		7	14	62	1916
Botryella		7	14	94	1916
Botryogene		7	15	259	1917
Botryosphaerostroma	Diplodia	32	62	302	1920
Calopactis		7	10	82	1910
Camarographium		16	34	306	1916
Caudosporella	Harknessia	53	123	135	1914
Ceratophoma	Sphaeronema	32	59	276	1917
Ceratopycnis	Hendersonia	53	124	86	1915
Chaetocytostroma	Fusicoccum	7	17	91	1919
Chondropodiella		32	59	261	1917
Cladochaete		7	10	318	1912
Collonaemella		53	124	82	1915
Columnothyrium		16	34	306	1916
Cornucopiella		53	124	118	1915
Cryptorhynchella	Sphaerographium	53	124	88	1915
Crytosphaerella		53	126	360	1917

Cytonaema	Cytospora	53	123	131	1914
Cytophoma	Cytospora	53	123	133	1914
Cytoplacosphaeria		7	17	79	1919
Cytosphaera		7	14	205	1916
Cytostagonospora	Stagonospora	7	14	150	1916
Cytotriplospora	Cytospora	59	7	47	1921
Dasypyrena		8	23	109	1912
Dasysticta		8	23	108	1912
Dearnessia	Stagonospora	32	58	25	1916
Diplodothiorella	Dothiorella	7	14	151	1916
Diploplacosphaeria	Thoracella	32	62	308	1910
Dothorellina	Dothiorella	16	29	70	1911
Dothisphaeropsis		53	128	616	1919
Ectosticta		8	23	107	1912
Endogleoea	Sirostomella	66	5	207	1915
Fumagospora		4	10	326	1911
Gamonaemella	Gamospora	49	6	123	1922
Haplosporidium	Pyrenochaete?	8	23	106	1912
Hemidothis		7	14	95	1916
Hendersonina		38	6	198	
Herpotrichopsis		53	123	115	1914
Jahniella		7	18	123	1920
Lasiostroma		54	27	472	1911
Leptophoma		53	124	73	1915
Lichenophoma	Dendrophoma	32	50	296	1910
Linochora		53	119	638	1910
Linchorella	Linchora	7	10	43	1912
Malachodermis		32	52	344	1912
Macrophomella	Macrophoma	7	14	63	1916
Mastigosporella	Harknissia	53	123	135	1914
Myriellina		53	124	100	1915
Myxofusicoccum	Fusicoccum	7	10	68	1912
Neohendersonia	Hendersonia	7	19	190	1921
Neoplacosphaeria	Placosphaeria	7	19	74	1921
Neosphaeropsis	Sphaeropsis	7	19	67	1921
Phacidiopycnis		67	22	147	1912
Phaeocytostroma	Cytospora	7	19	45	1921
Phellostroma		47	9	185	1914
Phyllostictina		7	14	185	1916
Placodiplodia		16	34	305	1916
Placonaemina		7	19	197	1921
Placophomopsis	Phomopsis	34	59	315	1921
Placothyrium	Cytosporina	16	34	302	1916
Plectonaemella		53	124	81	1915
Pleocouturea		4	10	326	1911
Pleosphaeropsis	Sphaeropsis	7	14	203	1916
Pleuronaema	Sphaeronema	32	59	257	1917
Pleurophoma	Dendrophoma	53	123	117	1914
Pleurophomella	Dothiorella	53	123	123	1914
Polyopeus	Phoma	43	58	239	1920
Pseudodiplodia	Diplodia	10	90	183	1920
Pseudohaplosporella		10	90	192	1920

Pycnis	Sclerophoma	53	123	129	1914
Pycnosporium	Cicinnobolus	26	51	515	1909
Pyrenochaetina		7	14	94	1916
Rhodoseptoria		54	29	178	1913
Sarcophoma	Sclerophoma	53	125	75	1916
Sclerochaetella	Plendomus	32	59	251	1920
Sclerophomella	Phoma	32	59	237	1917
Sclerophomina	Phoma	32	59	240	1917
Scleropycnis		7	9	278	1911
Sclerosphaeropsis	Sphaeropsis	5	28	209	1914
Sclerostagonospora	Stagonospora	32	59	252	1917
Sclerotheca	Camarosporium	57	11	314	1917
Sclerothyrium	Sclerophoma	32	60	181	1918
Scolecosporella	Hendersonia	7	19	30	1921
Septoriopsis	Septoria	41	11	4	1919
Sirophoma	Phoma	32	59	257	1917
Sirosperma		72	54	246	1916
Sirosphaera		47	8	502	1913
Sirostomella		53	125	75	1916
Sphaeriostromella	Phomopsis	16	34	297	1916
Sphaeronaemina	Sphaeronema	32	59	275	1917
Sphaerothyrium	Sclerophoma	16	34	299	1916
Steganopycnis		7	14	370	1916
Stenocarpella		7	15	258	1917
Subulariella	Sphaerographium	53	124	118	1917
Traversoa	Sphaeropsis	7	11	317	1913
Trotteria		87	10	54	1917
Vermiculariopsis	Vermicularia	20	10		1912
Verrucaster		2	21	383	1913
	Nectrioidaceae				
Blennoriopsis	Sirothyrella	7	17	92	1919
Cyanophomella		32	60	156	1918
Cyanochyta		53	124	92	1915
Dothiorina		53	120	464	1911
Gyrostroma		54	30	387	1914
Leptodermella		66	5	212	1914
Mycorhynchella		32	60	135	1918
Pycnidiella		53	124	93	1915
Plenozythia		7	14	215	1916
Scleropycnium		58	31	5	1912
Sirocyphella		53	119	650	1910
Stylonectria	(Fam. doubtful)	53	124	52	1915
Stylonectriella		53	124	53	1915
	Leptostromataceae				
Chaetopeltiopsis	Chaetothyrium	19	27	253	1913
Didymochora		32	60	172	1918
Diedickea	Pycnothyriaceae	7	11	268	1913
Discosiella	Discosia	36	5	1546	1912
Discothecium		7	14	371	1916
Ichnostroma		47	9	186	1914

Khekia		32	62	284	1920
Lasiothyrium		47	8	503	1913
Leptothyrina	Leptothyrium	53	124	123	1915
Massalongina	Leptostroma	16	34	319	1916
Peltaster	Asterostomula	7	15	261	1917
Phaeolabrella	Labrella	8	23	117	1912
Pirostomella		7	12	308	1914
Pleurothyrium	Leptostromella	16	34	322	1916
Pycnothyrium		7	11	175	1913
Rhabdothyrella		53	126	290	1917
Rhabdothyrium		53	124	125	1915
Rhizothyrium	Septothyrella	54	30	427	1914
Sirothyriella		53	119	451	1910
Sirothyrium		7	14	218	1916
Sphaerothyrium	Leptostroma?	16	34	298	1916
Thyriostroma		7	11	176	1913
Trachythyriolum		77	23	523	1918
	Pachystromaceae				
Hypodermina		53	125	55	1916
Microdiscula		53	124	142	1915
Pachydiscula	Scleropycnis	66	5	210	1914
Rhabdostromella		53	124	145	1915
Tryblidiopycnis		53	127	562	1918
Xenostroma		53	124	149	1915
	Excipulaceae				
Acleistia		59	5	420	1914
Acrosporium		16	29	385	1911
Bacterexcipula		32	60	161	1918
Chaetodiscula		32	50	44	1910
Desmopatella		16	37	159	1919
Dinemasporiella	Dinemasporium	32	52	385	1912
Excipuella	Excipula	53	124	109	1915
Exotrichum		7	12	571	1914
Falcispora		32	52	269	1912
Phaeopolynema	Excipula	8	23	117	1912
Psalidosperma		7	12	571	1914
Pseudolachnea	Pseudopatella	7	8	393	1910
Ramulariospora		5	28	216	1914
Stauronema		7	14	217	1916
Stictepatella		32	60	166	1918
	Melanconiales				
	Melanconiaceae				
Basilocula		7	12	210	1914
Cheiroconium		53	119	664	1910
Colletotrichella	Labrella	53	125	83	1916
Cryptosporiopsis		32	52	360	1912
Didmosporina	Chalara	53	125	83	1916
Discosporium		66	5	196	1914
Elaedemia		7	20	62	1922

Gloesporidiella	Gloeosporium	32	62	318	1920
Gloeosporidina	Gloeosporidium	7	19	214	1921
Heteroceras		7	13	136	1915
Marssoniella	Marssonina	53	125	108	1916
Myrioconium		7	10	448	1912
Stegosphaeria	Marsonia	7	14	362	1916
Titaespora		7	14	345	1916
Titaesporina		7	17	112	1919

MONILIALES

Moniliaceae

Amblyosporiopsis	Amblyosporium	49	6	127	1922
Beauveria	Spicaria	21	59	40	1912
Cristulariella	Cristularia	53	125	124	1916
Gemmophora		16	30	478	1912
Helicodendron		44	25	460	1912
Hormactinia		32	57	336	1915
Oosporoidea	Oospora	41	5	53	1913
Pachybasidiella		7	13	9	1915
Polymorphomyces		71	24	248	1914
Ramulispora	Ramularia	88	21	56	1921
Sporoclenia		12	7	302	1912
Triposporina		53	121	410	1912
Verticilliastrum	Verticilliopsis	22	24	302	1912

Dematiaceae

Casaresia		80	20	113	1920
Ceratosporella		16	37	154	1919
Chalaropsis		55	49	584	1916
Cheiropodium	Clasterosporium	7	13	42	1915
Columnophora	Chalara	7	14	349	1916
Cystodendron		7	12	212	1914
Dendryphiella		7	12	417	1914
Dichotomella		7	12	312	1914
Didymotrichum		53	123	139	1914
Endophragmia		54	36	86	1920
Eriomenella		44	25	447	1918
Harziella	Cladosporium	84	313	460	1916
Hormisciopsis	Hormiscium	41	6	32	1914
Lacellina		7	11	418	1913
Melanopsamella	Conytrichium	16	37	112	1919
Microbasidium	Haplobasidium	7	12	415	1914
Muiaria	Macrosporium	18	58	244	1914
Muiogone	Sporodesmium	18	58	239	1914
Phialophora		41	7	202	1915
Piricauda	Stigmella	7	12	218	1914
Pleurothecium	Acrothecium	16	37	154	1919
Septoidium		6	7	50	1921
Sirosporium	Macrosporium	32	52	278	1912
Stigmopsis	Stigmella	7	12	218	1914
Toruloidea	Torula	41	5	53	1913

	Stilbaceae			
Calostilbella		16	37	154	1919
Cladographium		44	25	439	1918
Coeleographium		24	2	13	1920
Coremiella		7	10	52	1912
Melanographium	Sporocybe	7	11	558	1913
Phaeostilbella	Stilbella	16	37	153	1919
Sporostachys		87	10	54	1917
Stilbodendron		7	14	260	1916
Synnematium	Hirsutella	41	12	74	1920
	Tuberculariaceae			
Amphichaete	Chaetospermum	53	123	142	1914
Anomomyces		16	37	153	1919
Cheiromycella		55	119	864	1910
Clathrococcum	Epicoccum	53	120	473	1911
Discofusarium		59	7	164	1920
Exosporella		53	121	414	1912
Leucodochium		7	15	266	1917
Marcosia		7	14	96	1916
Periolopsis		9	11	357	1913
Petrakia		7	11	407	1913
Phanerocorynella		16	37	157	1919
Sigmatomyces		7	11	319	1913
Sirodochiella		7	19	144	1921
Thyrostroma	Epicoccum	53	120	472	1911
Tubercularielle		66	5	209	1914
Verticilliodochium	Tubercularia	7	12	220	1914
Xiphomyces		7	14	374	1916

The following fungi are of UNKNOWN AFFINITY.

	Graphiolaceae			
Stylina		7	18	192	1920
	Coccoidaceae			
Coccodiella		19	25	222	1911
	ASCOMYCETES			
Haplostroma		7	14	80	1916
Konenia		19	27	250	1913
Melanomyces	Pseudoparodia	7	15	196	1917
Miyakeamyces		19	27	248	1913
Solanella		40	3	268	1910
	FUNGI IMPERFECTI			
Chlamydosporium		33		18	1913
Gloeodes		60	13	157	1920
Menezesia		20	9		1913
Nothospora		33	20		1913
Saprophorum	(Hyphomycete)	72	54	246	1916
Sirostomella		53	125	78	1916
Spirospora		54	36	96	1920

Stenocarpella		7	15	258	1917
Trichodiscula		51		73	1910
No knowledge as to position					
Alichora		16	37	158	1915
Amphoromorpha		18	58	249	1914
Candelospora		50	31		1911
Canthransiopsis		18	58	247	1914
Cryptoacsus		56		114	1911
Diploospora		34	54	220	1916
Ephelidium		10	90	184	1920
Fusicladiella	Carlia	16	37	155	1919
Heptasporium		63	18		1912
Mitopeltis		77	23	93	1918
Neofabraea	Mollisiaceae	17		178	1911
Parendomyces		25		374	1910
Peltomyces		27	149	239	
Pericystis		9	26	798	1912
Phaeocryptopus	Cryptopus	79	30	424	1914
Plenophysa		7	17	142	1919
Rachisia		30	17	465	1913
Rachodiella		55	52	21	1919
Xenopeltis		7	17	39	1919
	Mycelia Sterilia				
Graillomyces		18	65	245	1918

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DEPARTMENT OF METHODS, REVIEWS, ABSTRACTS, AND BRIEFER ARTICLES

HEMISTOMUM CONFUSUM, A HOMONYM

By
John E. Guberlet
Parasitologist, Oklahoma Agricultural Experiment Station

Dr. Maurice C. Hall recently called the attention of the writer to the use of a homonym in connection with the description and naming of a new species of holostome, *Hemistomum confusum* Guberlet, 1922 (Jour. Parasit., 9:6-14). The specific name *confusum* is a homonym of a species described by Krause, 1914, (Ztschr. f. Wissensch. Zool., Leipz. & Berl., 112:93-238), whose work on holostomes was unknown to me during my investigations. Therefore, a new specific name must be submitted for my species, for which I propose *indistincta* in place of *confusum*.

Hall and Wigdor, 1918 (Jour. Am. Vet. Med. Assoc., 53:616-626), whose work was overlooked by me, were also apparently unaware of Krause's work but arrived at the same conclusion as Krause relative to the nomenclature of the genus *Hemistomum* Diesing, 1850. In both works it is shown that the name *Hemistomum* is not in good standing and that the name *Alaria* Schrank, 1788, is the correct generic term with *alata* Goeze, 1782, as the specific name for the type species. Therefore, according to priority, the combination should be *Alaria alata* (Goeze, 1782) Krause, 1914.

In view of the foregoing and following the suggestion of Hall by communication, I wish to substitute the generic name *Alaria*, in place of *Hemistomum*, for the holostomes recently described by me (1922), i.e. *H. gavium* and *H. indistincta*. Hence the substitution will change the names to *Alaria gavia* and *A. indistincta*.

THE USE OF SODIUM SILICATE AS A MOUNTING MEDIUM

By
Charles W. Creaser and William J. Clench
Museum of Zoology, University of Michigan

In preparing slides for use in two widely different fields the writers have had occasion to use a solution of sodium silicate (water-glass) as a mounting medium. Several investigators have used this substance but there seems to be no literature on the subject. Mr. W. F. Clapp of Cambridge, Massachusetts, has used it in mounting the radulae of certain mollusks. Through a knowledge of his use of sodium silicate as a mounting medium, the present development had its inception. Several investigators have inquired about the methods best adapted for working with it, and its availability for their problems. Our experience with this substance is made known here.

Sodium silicate is soluble in water, and basic in reaction, therefore, it can only be used with basic stains. Fish scales may be mounted as total objects from water without staining. In mounting radulae of certain fresh water mollusks, basic fuchsin has given very good results.

For the stock of sodium silicate, we have used that sold by the ordinary drug store for preservation of eggs. We find it as good as any of the more carefully prepared solutions. Care must be used in the selection of the stock since this substance not uncommonly contains a fine white precipitate in suspension. This kind of material produces a mount with a milky or clouded appearance. Sodium silicate may be kept in the ordinary balsam bottle, but it should not be stored in glass-stoppered bottles.

The ordinary water solution of sodium silicate, in its commercial form, may be used as described here. It has, however, certain defects that render the slides unfit for use after approximately three months. We have used it to prepare mounts that are to be studied in a vertical position. By the use of this material, solid, durable mounts may be produced which do not melt or run when heated and in a very short time may be used in a vertical position. It is for this specific purpose that we have found sodium silicate very useful. Objects mounted in glycerine-jelly will not stay in position under the influence of heat and gravity until after they have been prepared for some time. Glycerine jelly and similar media have a tendency to clear, but it is necessary to avoid this effect in fish scales and mollusk radulae. In field work where it is difficult

to make mounts with a medium that must be heated the use of a cold solution of sodium silicate has much in its favor. Optically, these slides are excellent in revealing the characters of fish scales.

It has been found that mounts made of the commercial solution of sodium silicate are not permanent since this medium will crystallize in three or four months. However, this difficulty seems to have been overcome by the use of glycerine in connection with the sodium silicate. Our slides, made according to this revised formula, have not been kept long enough to show what the ultimate outcome will be, but after several months they show no tendency to crystallize.

The fish scales are placed in water, cleaned, and allowed to soak for a time. They are then transferred to clean water or to dilute solutions of glycerine or of sodium silicate. Mounting from either water or glycerine into sodium silicate has been found very satisfactory. Care must be used that the object is free from all alcohol as a trace of this will cause a white precipitate to form over the object when it is placed in the sodium silicate. It has been our practice to put the sodium silicate solution on the slide by the use of a solid glass rod or to pour it direct from a small bottle. The medium should be spread over most of the area to be covered by the cover glass. The objects are then transferred from the water or the dilute solutions of water-glass or glycerine before they dry and placed in the desired position on the slide. Objects may be placed on the slide first in a small drop of water, the water-glass and the cover glass being put on afterwards.

Since the water solution of sodium silicate quickly forms a tough film over the exposed surface much care and speed must be used in placing on the cover glass, in order to prevent air bubbles and to insure the spreading of the medium to all parts of the area under the cover glass.

The slides are allowed to set. Air bubbles will work their way out if the slide is at a very slight angle during this time. These slides will set in two or three days and can then be used in a vertical position and the objects will not be displaced by heat or gravity.

Cleaned radulae are transferred directly from water to a water soluble, basic stain and then returned to water where the excess stain is removed. They are then mounted directly in a drop of water-glass in the same manner as are the fish scales. Ringing the slides in the ordinary manner will insure greater permanence in these mounts.

Glycerine sodium silicate may be prepared as follows: To a solution of commercial sodium silicate, slightly diluted glycerine is added and the liquids mixed by shaking. The proportions may vary but the best results are obtained by using twelve parts of water-glass to one part of glycerine. If the mixture does not go into a homogeneous solution a little water should be added. This medium does not set as rapidly as the sodium sili-

cate solution and is therefore easier to handle. Objects may be mounted in it in the manner described above. An excess of medium may be removed with warm water and discarded slides placed in water may be easily cleaned after a few hours. Slides prepared from this medium are excellent for the study of fish scales and mollusk radulae. They seem to be quite permanent.

NEW POCKET DISSECTING MICROSCOPE

E. G. Campbell of Purdue University has described and illustrated (Science, 1923, 57:179-180) a simple, efficient instrument for the examination of small objects in the field. Focusing, rotation of the object, and dissection may all be performed simultaneously and with ease as the microscope is held in the hands of the observer. The pocket adjustment of the instrument provides not only for concealment and protection of the working parts but also storage space for dissecting instruments. Change from pocket to working adjustment is simple and quickly accomplished. Two figures show clearly the details of construction of this instrument.

THE PHYSIOLOGY OF REPRODUCTION, by F. H. A. Marshall. Second revised edition; 770 pages, 189 illustrations. Longmans, Green and Co., London and New York, 1922. Price $12.00.

Since Dr. Marshall's "Physiology of Reproduction" first appeared it has been the standard and very useful text and reference book in its field. The excellent arrangement of its materials, its brevity of statement, and the clearness and cogency of its summaries have made it one of the most satisfying and dependable reviews of knowledge in any field of comparative biology. The manner of handling the references to original papers is adequate, and gives a historical view without being overwhelming.

The new edition loses none of the admirable qualities of the first and brings the survey up to date by recording the progress of the last twelve years. Aside from such detailed revision certain large topics have been rewritten and elaborated. Some of these are: The Biochemistry of the sexual organs; Changes in the maternal organism during pregnancy; Fertilization; Internal secretory functions of the reproductive glands; Sex-determination, and the causes of birth. A special discussion is included of Child's theory of rejuvenescence and senescence as these bear upon certain types of reproductive and life cycles not so well explained by the theory of the continuity of germplasm and the segregation of germ cells.

Aside from its indispensable use to all teachers and students of biology, zoology, and physiology, the book is of high value to gynecologists, veterinarians, and animal breeders. Subject and author's indices are excellent; the printing, illustrating, and mechanical appearance are worthy of the matter.

T. W. Galloway

MARY ALLARD BOOTH

September, 8, 1843 **September, 15, 1922**

Never has a family motto been typified so truly as in the life of Mary Allard Booth. "What I hope to accomplish I shall accomplish" seems to have been the daily inspiration which aided her, in spite of many obstacles, to the highest achievement in the labors undertaken.

Born at Longmeadow, Massachusetts, on September 8th, 1843, the years of her childhood and young womanhood were a record of ill health and affliction, and during her early education in the local schools, much of the work and play of ordinary youth were denied her.

In speaking of her family, Miss Booth said, "My mother's people were a literary family, the Bartons of Vermont, of whom Clara Barton, President of the Red Cross, was one. From my father I inherited my love of science."

During a visit to the shores of Long Island Miss Booth's attention was first directed to the interesting forms of aquatic life. While seated in her wheel chair she watched a woman near by who was intent upon gathering sea weeds. The woman was Miss Mary Halliday of Brooklyn, and during the chance acquaintance which the occasion offered, Miss Halliday took pleasure in explaining to the invalid girl the wonderful structure and life history of some of the marine algae.

This incident seemed to be just what was needful to awaken the interest which her father had long sought to arouse, and with his help and knowledge at her constant disposal she began the work which was to fill her long life with an unfailing enthusiasm and which by painstaking application placed her at last in the foremost rank of scientific workers.

In 1877 she purchased her first compound microscope and step by step advanced into the wonderland which it disclosed. The delicate manipulation necessary in the work, the skill in preparation and mounting of specimens were the result of long and patient endeavor due to love of the work. No object in the field of nature was too minute for careful study. When the wonders of this new world were thus revealed to her, Miss Booth sought some method by which she could make this microcosmos intelligible to others and there the camera seemed to fill the required need.

With microscope and camera, then, she was able to make of vast importance to humanity the line of study so assiduously pursued. When the invasion of bubonic plague seemed imminent in this country Surgeon

General Blue turned to Miss Booth with a request for photomicrographs of the plague fleas infesting rats in San Francisco, and these photographs were used in a nation-wide lecture campaign for the extermination of the plague. Not only this Government but those of France and England as

Mary A. Booth

well sought her aid in the fight against such subtle enemies. Many infinitesimal creatures were sent on the long journey to her laboratory in Springfield, Massachusetts, there to be photographed and studied scientifically.

All the steps in the painstaking process from the preparation of the object, proper staining to emphasize structural detail, delicate mounting on microscopical slide, photographing, developing the negative and making the prints, were performed by this patient worker, and each picture was a masterpiece of its kind. And in the midst of all this patient endeavor and accomplishment, Miss Booth found time to make for herself an honored place in the lecture field and editorship of several scientific journals.

The great esteem in which she was held at home and abroad is evidenced by the honors conferred upon her. Few women have shared with her the distinction of fellowship in the American Association for the Advancement of Science, and she was likewise one of the few women elected a fellow of the Royal Microscopical Society of London. As a higher reward for her humanitarian services the privilege was given her to continue her activity in full strength and vigor of mind up to the very close of her life.

Her latest photographs were those of a family of busy little squirrels who made their home in a neighboring tree and who had learned to know and love the good friend who cared for them so faithfully. Indeed, as was a fitting close for such a lover of nature her last act on earth was the carrying of the evening meal to these little creatures; and in the great out-of-doors, at the close of a September day, surrounded by these appreciative friends, she passed into the great beyond.

BESSIE PERRAULT TITUS

PROCEEDINGS OF THE AMERICAN MICROSCOPICAL SOCIETY

Minutes of the Boston Meeting

The 41st annual meeting of the American Microscopical Society was held in affiliation with the American Association for the Advancement of Science at Boston, Mass., December 28, 1922.

President N. A. Cobb presided at this meeting.

The report of the Treasurer for the year 1922 was read by the Secretary and was referred to an Auditing Committee composed of Professors F. H. Krecker and J. W. Kostir.

The report of the Custodian was read by the Secretary and was referred to an auditing committee composed of Messrs. Edw. Pennock and F. E. Ives.

The meeting voted to send hearty congratulations to the Custodian on the growth of the Spencer-Tolles Fund.

The Secretary presented a report on the affairs of his office, this report covering the previous three years.

The following officers were nominated and elected: President, Professor Chancey Juday, University of Wisconsin, Madison, Wis.; First Vice-President, Dr. B. H. Ransom, Bureau of Animal Industry, Washington, D. C.; Second Vice-President, Dr. W. W. Cort, Johns Hopkins University, Baltimore, Md.; Secretary, Professor Paul S. Welch, University of Michigan, Ann Arbor, Mich.; Treasurer, Dr. Wm. F. Henderson (for two years), University of Pittsburgh, Pittsburgh, Pa.

Professor Geo. R. La Rue, University of Michigan; Professor Z. P. Metcalf, North Carolina State College of Agriculture and Engineering, and Professor E. M. Gilbert, University of Wisconsin, were chosen as the elective members of the Executive Committee for 1923.

Dr. B. H. Ransom, Bureau of Animal Industry, was chosen as a representative of the Society on the Council of the American Association for the Advancement of Science.

Dr. N. A. Cobb, Bureau of Plant Industry, was appointed as a member of the Spencer-Tolles Fund Committee.

Adjourned.

Paul S. Welch, *Secretary.*

CUSTODIAN'S REPORT FOR THE YEAR 1922

Spencer-Tolles Fund

Balance reported for the year 1921		$9104.56
Interest on Bonds	250.00	
Dividends Penna. R. R. Co.	96.75	
*Build'g. & Loan Ass'n	144.16	490.91
		9595.47
Less Grant to Wm. P. Hayes		60.00
		9535.47
Net Increase during the year	$430.91	

*Estimates, proved correct by letter of B. & L. Ass'n. of Dec. 19-'22. M.P.

TOTALS

Receipts

All contributions		802.03	
All sales		1193.38	
All life memberships		300.00	
All interest, dividends, profits		7590.06	
		9885.47	
Disbursements			
All Grants	310.00		
All life membership dues	40.00	350.00	9535.47

INVESTMENTS

Stock in Keystone State Bldg. & L. Ass'n	2385.47	
Bonds, Rio Grande Junction R'y	5000.00	
Stock, 43 shares Penna. R. R. Co	2150.00	9535.47

Life members: (Robert Brown, dec'd.); J. Stanford Brown, Seth Bunker Capp, Harry B. Duncanson, A. H. Elliott (dec'd.) and John Hately (dec'd.).

Contributions of $50 and over: John Aspinwall, Iron City Microscopical Society, Magnus Pflaum and Troy Scientific Society.

(signed) MAGNUS PFLAUM
Custodian

Philadelphia, Pa.
Dec. 30, 1922

Philadelphia, Feb. 1*st* 1923

Having examined the above account, and the securities on hand as shown therein, we find them correct.

F. E. IVES
EDWARD PENNOCK

ANNUAL REPORT OF THE TREASURER OF THE AMERICAN MICROSCOPICAL SOCIETY

December 24, 1921 to December 13, 1922

RECEIPTS

Balance on hand, December 24, 1921	$ 592.71
Dues received for Volume 40 or before	45.00
Dues received for Volume 41	170.10
Dues received for Volume 42	252.00
Dues received for Volume 43	2.00
Initiation Fees	24.00
Subscriptions for Volume 40 or before	3.00
Subscriptions for Volume 41	315.02
Subscriptions for Volume 42	54.00
Sales of Transactions, duplicates, back numbers	26.21
Advertising, Volume 39	10.00
Advertising, Volume 40	250.00
Authors, for preparation of plates	34.30
Grant, from Spencer-Tolles Fund	60.00
Sundries	.50
Total	$1838.84

EXPENDITURES

Printing Transactions, Volume 40, No. 4	$ 224.02
Printing Transactions, Volume 41, No. 1	257.16
Printing Transactions, Volume 41, No. 2	256.77
Printing Transactions, Volume 41, No. 3	200.05
Postage and Express for Secretary	21.00
Postage and Express for Treasurer	13.00
Office expenses of Secretary	47.82
Office expenses of Treasurer	28.65
Secretary, trip to Toronto	39.89
Balance on hand	750.48
Total	$1838.84

December 13, 1922.

W. F. HENDERSON, *Treasurer.*

Report of the Auditing Committee of the American Microscopical Society

The accounts of W. F. Henderson, Treasurer of the American Microscopical Society, for the period beginning December 24, 1921, and ending Dec. 13, 1922, have been examined by the Auditing Committee and have been found to be correct.

Respectfully submitted,

W. J. KOSTIR

F. H. KRECKER

Feb. 28, 1923.

TRANSACTIONS

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79

THE DISTRIBUTION OF FROG PARASITES OF THE DOUGLAS LAKE REGION, MICHIGAN[1]

By
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University of Vermont

Introduction

During the summers of 1917 and 1919 the writer made a study of the parasites of frogs and their distribution in the Douglas Lake Region, Cheboygan County, Michigan. It was thought that a study of the distribution of frog parasites, including detailed statistical data, might present some interesting as well as valuable information. Three species of frogs were examined within a limited area, thus furnishing data contributing to the geographical distribution of frog parasites.

In all, two hundred eight hosts were examined from eleven local stations (See Map Fig. 1.). The collections were made during the months of July and August of the two years, 1917 and 1919. Some differences occurred in the two seasons which are worth noting and these will be stressed in the discussion. The particular habitats from which hosts were taken are described below.

Bryant's Bog is a typical bog association with plant life slowly encroaching upon the water. As a matter of fact, very few frogs inhabit this place. So few frogs were taken there that a lengthy description of the habitat is not warranted.

Carp Creek is a short, rapid, spring-fed trout stream. The majority of specimens taken from this habitat came from the roadside among the grass which grew around two large moss-covered logs. A few were taken from an abandoned road along the stream where mosses and liverworts were abundant. On account of the swift current, breeding could take place here only in the side pools. In 1919, but two specimens were taken in this locality from the roadside. The vegetation of the abandoned road was dense and practically no open places existed as in 1917.

Fairy Island furnished but one specimen each season. Here the shore and bottom consisted mostly of gravel. Sedges were the only vegetation

[1] Contribution from the University of Michigan Biological Station.

along shore, but they afforded resting places for several species of Coleoptera, Hemiptera, and Hymenoptera, which served as food for the frogs.

Lancaster Lake is a small body of water near Douglas Lake, having an area of several acres. A small stream, Bessey Creek, connects Lancaster Lake with Douglas Lake, thus presenting a very easy path of migration for frogs from one lake to the other. The shore was thickly matted with

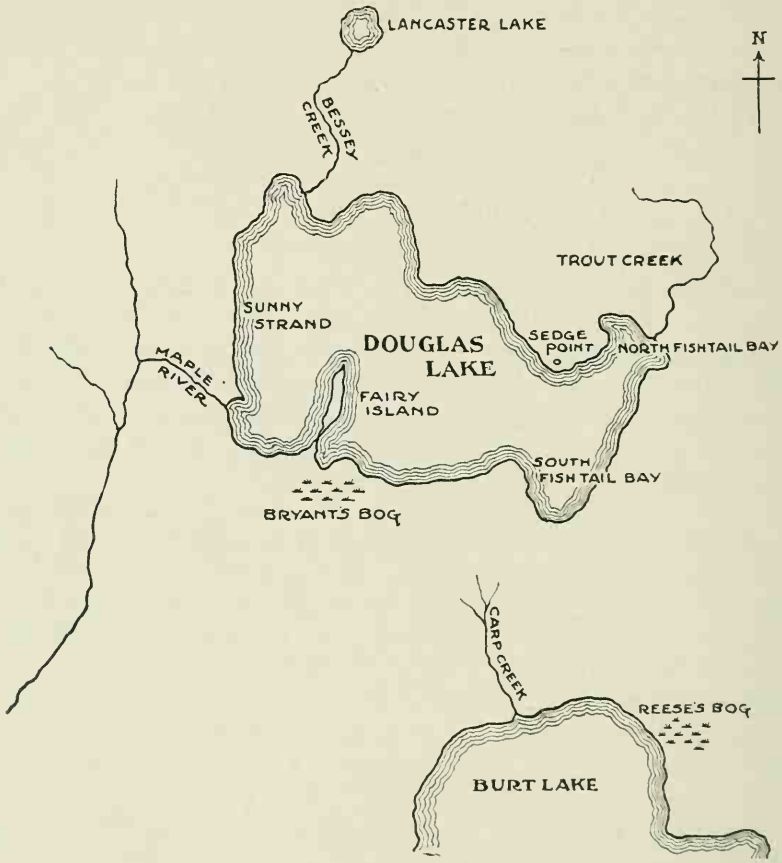

Fig. 1. Collecting Stations

sedges. Small bushy willows provided a shaded shelter between the sedges and the edge of the wooded area. It was here that most specimens were taken. The soil between the water and the wooded area consisted mostly of muck. Small depressions were present in which numerous aquatic insects thrived. There was practically no visible change in this habitat since 1917.

Maple River, the outlet of Douglas Lake, runs through a comparatively level stretch of country. Scattered along its course are numerous bayous

affording excellent breeding places for frogs. Sedges and grasses were very dense and stumps and roots of trees lined the banks. Cat-tail societies were also present. The bottom was muddy or sandy, and at places stones and gravel were prevalent. Deep holes and shallows were common.

North Fish Tail Bay (N. F. T. Bay in tables), an arm of Douglas Lake, presents a habitat consisting of a level sandy shore with a thick growth of sedges. The bottom consists of sandy marl and muck. A few potomogetons and some chara are present in the water. Here the frogs have access to many species of insects and some few snails.

Trout Creek is a small trout stream rising in bogs to the north and east of Douglas Lake. The vegetation along this stream is varied. Typical roadside plants and trees are present along the banks of the stream. Small areas of water lilies occur in quiet stretches of water. Algae are also present. Grasses overhang the water in many places. At some places the bottom consists of muck; at others gravel forms the main constituent of the bed of the stream. The stream is swift in some few places; at others it is slowly running or even stagnant. Some places are in dense shade and others in bright sunlight. The depth of the water varies from a few inches to a foot and a half.

Reese's Bog is a large Thuja bog on the north shore of Burt Lake. Conditions in this bog had undergone considerable change between the years 1917 and 1919. At one place where many specimens were taken in 1917 moisture was lacking in 1919 and very few frogs were taken. At another place the grass had become very dense and it was too dry for a frog habitat. In still another area certain mosses and grasses had become denser, thus holding back more of the surface drainage which made the habitat too moist for certain kinds of insects. On slightly higher ground conditions were more favorable but frogs were not as abundant as in 1917. The *Rana clamitans* which were taken here were found near the water and in secluded and well sheltered spots. *Rana pipiens* wanders a considerable distance from the stream in contrast to *Rana clamitans*, which is always found near the main body of water.

Sedge Point is situated on the north shore of Douglas Lake, not far from the western margin of North Fish Tail Bay. Here several pools are cut off from the lake as a result of wave action. Around these pools there was a zone of water plants. One pool usually containing water was devoid of water in 1919, but the soil was somewhat moist. Here insect life was very abundant. Snails, too, were very numerous, eight species being present; these no doubt figuring largely in the life histories of many of the parasites.

South Fish Tail Bay (S. F. T. Bay in tables) is the site of the Biological Station. Here the shore is sandy. There is a very scant growth of plants in the water, and but few sedges along the shore. Very few frogs make this a permanent habitat.

Sunny Strand is a sedge-covered beach situated at the north-western part of Douglas Lake. The water along this shore is very shallow for fifteen or twenty yards out. On account of the direction of the prevailing winds this is a sheltered shore. No hosts were taken from this place in 1917, but in 1919 four were taken.

Discussion

Three species of hosts were examined, one hundred seventy-seven specimens of *Rana pipiens*, twenty-nine *Rana clamitans*, and two *Rana cantabrigensis*, making a total of two hundred eight. No *Rana catesbiana* were seen at any time during the two summers, altho they are recorded for the region. Of the number collected, seven individuals, all of small size, seemed to be entirely free from parasites. A total of ten species, excluding the nematodes, parasitic in frogs were found. Six, however, was the highest number of species found in any individual host. The nematodes are in the hands of an expert and their distribution will have to be reported at a later date.

It might be interesting to note that when frogs were fed daily in captivity, then examined, no loss of intestinal and urinary bladder parasites were noted. Loss occurred, however, where no feeding was done within 12-24 hours, and the parasites were recovered from the bottom of the aquarium.

Table No. I shows the number of hosts taken from each habitat in each of the two seasons.

Table No. I

Number of Hosts Taken from Each Habitat

	R. pipiens		*R. clamitans*		*R. cantabrigensis*	
	1917	1919	1917	1919	1917	1919
Bryant's Bog	3	.....	1	.....	.....	.....
Carp Creek	19	2	1	.....	.....	.....
Fairy Island	1	1	.....	.....	.....	.....
Lancaster Lake	7	12	1	.....	.....	.....
Maple River	11	28	16	6	.....	1
N. F. T. Bay	1	7	.....	.....	.....	.....
Trout Creek	4	3	.....	1	.....	.....
Reese's Bog	20	22	1	2	.....	.....
Sedge Point	1	20	.....	.....	.....	1
S. F. T. Bay	9	2	.....	.....	.....	.....
Sunny Strand	.....	4	.....	.....	.....	.....
Total collected	76	101	20	9	.....	2
Grand total	.....	.....	.....	.....	.....	208

Table No. II shows the parasites found and their local distribution.

TABLE No. II

Parasites Found and Habitat Distribution

	Bryant's Bog	Carp Creek	Fairy Island	Lancaster Lake	Maple River	North Fish Tail Bay	Trout Creek	Reese's Bog	Sedge Point	South Fish Tail Bay	Sunny Strand
Octomitus intestinalis Prowazek	x	x	x	x	x	x	x	x	x	x	x
Opalina obtrigonoidea Metcalf (An as yet unpublished species)	x	x	x	x	x	x	x	x	x	x	x
Nyctotherus cordiformis Stein	...	x	x	x	x	x	x	x	x	....	...
Diplodiscus temperatus Stafford	...	x	...	...	x	x	x	x	...	x	...
Gorgoderina attenuata Stafford	x	x	x	x	x	x	x	x	x	x	x
Pneumoneces medioplexus Stafford	x	x	x	x	x	....	x	x	x	x	x
Cephalogonimus americanus Stafford	...	x	...	...	...	....	...	x	...	....	...
Clinostomum attenuatum Cort	...	x	...	...	...	....	...	...	...	x	...
Proteocephalidae	...	...	...	...	x	....	...	...	...	x	x
Pneumoneces similiplexus Stafford	...	...	...	...	x	....	...	...	...	....	...

The identification of trematodes was confirmed by Dr. W. W. Cort; the *Opalina* by Dr. M. M. Metcalf; and the other forms by Dr. G. R. LaRue.

Octomitus was present in the *Rana cantabrigensis* from Maple River, and *Opalina* and a lung nematode were found in the host from Sedge Point.

Octomitus and *Opalina* were present in all localities. *Nyctotherus* was not secured in frogs from three habitats, probably due to the small number of hosts taken. Specimens of *Diplodiscus* were not taken from five of the eleven localities. Their absence from collections made at Lancaster Lake and Sedge Point is not to be explained on the basis of small numbers of hosts examined since large numbers of hosts were taken from those localities. *Gorgoderina* seems to be evenly distributed. The heaviest infection of this parasite noted was fourteen from a single specimen. Specimens of *Pneumoneces medioplexus* were taken from all localities except North Fish Tail Bay. There is a possibility of its being present there also, as but eight hosts were examined from that region. The heaviest infection with this species in any one frog was from the Maple River habitat, one lung containing thirty-eight, the other thirty-four. Another host from Sedge Point had thirty-six in one lung and thirty-four in the other. *Pneumoneces similiplexus* was taken only in the one locality, Maple River. Six specimens of *Cephalogonimus* were taken during the two summers from three hosts and two localities. Since these two localities

are not far apart it would be possible for hosts to migrate readily from one of these habitats to the other in a short time, and thus account for its presence in both places. *Clinostomum attenuatum* was taken in the same year, 1917, from but two hosts, which were very heavily infested. *Proteocephalidae* were obtained from three stations from both *Rana pipiens* and *Rana clamitans*.

Protozoan parasites alone infested the very small frogs which leads one to believe that the frogs become infested with the metazoan parasites when they feed on animal life. An exception to this fact, however, is that Dr. L. R. Cary found *Diplodiscus temperatus* in tadpoles. Any one of the protozoans, *Opalina*, *Nyctotherus*, and *Octomitus*, does not seem to be inconvenienced by the presence of the other forms, as nine hosts examined contained hundreds of all three species. One of the striking things about the Protozoa is the fact that Opalinae were found in only one specimen of *Rana clamitans* of the twenty-nine examined, while the percentage of infestation of *Rana pipiens* with this organism is 61 and 80 for the two summers respectively. Why the infestation differs in the two species is a question that cannot be answered until more examinations have been made paying particular attention to the other inhabitants of the habitat which may serve as food and act as carriers. Dr. R. W. Hegner, in an article published since these notes were written, has pointed out that while the tadpoles of *Rana clamitans* are infected with Opalinae the adults very rarely are infected.

A sufficient number of frogs were not collected at the Sedge Point locality in 1917 to make a fair comparison with those collected during 1919. Collections made at this habitat might shed some light on the adult forms of the cercariae present there. Extensive studies have been and are being made on the cercariae of this habitat. For instance no *D. temperatus* were found there, and we might conclude if larger numbers of hosts were examined and none found that the cercariae of that form do not exist there.

In all the examinations no Acanthocephali were found. This corroborates statements of other investigators who have found a very limited number upon examination of similar hosts in North America.

A comparison of the species of the parasites found in *Rana pipiens* and *Rana clamitans* is made in Table III.

As mentioned before, it is a remarkable fact that *Rana clamitans* is so lightly infested with Opalina. Diplodiscus, *Pneumoneces medioplexus*, Cephalogonimus, and at least three species of nematodes were present in *Rana pipiens* and not found at all in *Rana clamitans*. Another striking comparison is the relatively low infestation of *Rana pipiens* with *Pneumoneces similiplexus* and Proteocephalidae as compared with that of *Rana clamitans*.

TABLE No. III

Comparison of Infestation of Two Species of Frogs Expressed in Percentages of Infested Individuals to Entire Number Examined

	Rana pipiens	*Rana clamitans*	*Rana pipiens*	*Rana clamitans*
	1917		*1919*	
Octomitus	30	18	48	88
Opalina	61	5	80	0
Nyctotherus	2	15	22	22
Diplodiscus	30	0	1	0
Gorgoderina	51	50	38	66
Pneumoneces medioplexus	5	0	30	0
Pneumoneces similiplexus	1	20	0.9	11
Cephalogonimus	3	0	0	0
Clinostomum	2	0	0	0
Proteocephalidae	0	10	1	0

As is shown in Table IV there is not enough variation in infection between the sexes to justify the consideration of each sex separately. The males and females live under the same conditions and in the same habitat, and, as would be expected, the percentage of infection between the two sexes does not differ to any great extent.

TABLE No. IV

Percentage of Infection of Total Number of Frogs (112) Collected During the Year 1919

	57 Females	55 Males	112 Males and Females
Opalina	70	74	72
Nyctotherus	19	25	22
Octomitus	50	52	51
Bladder flukes	36	43	39.5
Lung flukes	28	27	27.5
Diplodiscus	3	0	1.5
Cephalogonimus	1	0	.5
Proteocephalidae	1	0	.5

Comparisons of the percentage of infestation of both sexes of the same locality also give similar results.

The comparison of the percentage of infection of the hosts of the two summers in the various collecting places shows but slight variation except where a habitat was affected by drought or other factors. Variation is slight particularly where a large number of hosts were taken and can readily be seen in Table V.

TABLE V

Comparing Percentage of Infestation of Two Summers in the Various Localities

		Bryant's Bog	Carp Creek	Fairy Island	Lancaster Lake	Maple River	North Fish Tail Bay	Trout Creek	Reese's Bog	Sedge Point	South Fish Tail Bay	Sunny Strand
Octomitus intestinalis	'17	50	5	0	42	23	100	50	38	100	33	...
	'19	...	0	100	33	65	14	50	20	80	100	75
Opalina obtrigonoidea	'17	50	45	100	85	38	100	50	57	100	44	...
	'19	...	50	100	83	57	85	75	79	76	0	100
Nyctotherus cordiformis	'17	0	5	100	0	11	0	25	9	100	0	...
	'19	...	0	100	25	2	14	75	25	28	0	0
Diplodiscus temperatus	'17	0	30	0	0	3	0	25	33	0	66	...
	'19	...	0	0	0	0	14	0	0	0	0	0
Gorgoderina attenuata	'17	50	65	100	18	42	0	75	47	0	55	...
	'19	...	50	0	16	37	28	0	29	42	100	50
Pneumoneces medioplexus	'17	50	0	100	0	0	0	0	4	0	11	...
	'19	0	50	0	8	41	14	0	4	42	50	50
Pneumoneces similiplexus	'17	0	0	0	0	19	0	0	0	0	0	...
	'19	...	0	0	0	2	0	0	0	0	0	0
Cephalogonimus americanus	'17	0	10	0	0	0	0	0	4	0	0	...
	'19	...	0	0	0	0	0	0	0	0	0	0
Clinostomum attenatum	'17	0	5	0	0	0	0	0	0	0	11	...
	'19	...	0	0	0	0	0	0	0	0	0	0
Proteocephalidae	'17	0	0	0	0	7	0	0	0	0	11	...
	'19	...	0	0	0	0	0	0	0	0	0	25

An abundance or lack of food appears to be a factor of considerable importance. The more a host eats the greater opportunity it has to become infected. Abundance of food for frogs depends upon abundance of rainfall and vegetation. Among the stomach contents of the hosts examined were noted adults of the following orders of Insects: Hemiptera, Orthoptera, Coleoptera, Lepidoptera, and Hymenoptera. Representatives of the following groups of Coleoptera were recognized: Chrysomelidae, Scolytidae, Prioninae, and Cicindelidae. Larval forms of Hemiptera, Coleoptera, Lepidoptera, Hymenoptera, and Diptera were also found.

Conditions of the habitats at Lancaster Lake and Maple River remained practically the same from 1917 to 1919, and no great differences were found in the percentage of infection by comparing the two summer's finds. *Pneumoneces medioplexus* seems to be the exception here. At Carp Creek the frogs were not so abundant in 1919 as in 1917, and the vegetation was very different. With two exceptions, the infestations of Opalinae and Gorgoderinae in frogs from the Carp Creek habitat, were markedly different in the two seasons. Many species found in 1917 were not present in 1919.

Table VI gives for each parasitic species the percentage of the entire number of frogs infested. As can be seen, the heaviest infestations were with *Octomitus*, *Opalina*, and *Gorgoderina*.

TABLE No. VI

Percentage of Infestation of Entire Number of Frogs (208) Examined, 1917 and 1919

	112 Females	96 Males	208 Males and Females
Octomitus intestinalis	37	44	40.5
Opalina obtrigonoidea	58	66	62
Nyctotherus cordiformis	15	17	16
Diplodiscus temperatus	13	10	11.5
Gorgoderina attenuata	47	42	44.5
Pneumoneces medioplexus	13	20	16.5
Pneumoneces similiplexus	2	2	2
Cephalogonimus americanus	2	0	1
Clinostomum attenuatum	0	2	1
Proteocephalidae	3	0	1.5

Seasonal differences probably are due to a variety of factors. The amount and time of rainfall certainly affects the presence of parasites in a given locality in any one year.

Table VII gives the amount of rainfall and the temperature previous to and during the collecting periods of both years.

TABLE VII

RAINFALL AND TEMPERATURE

MAY	1917	1919
Rainfall	3.53 inches	3.49 inches
Maximum temperature	77° F.	83° F.
Minimum temperature	29° F.	31° F.
JUNE		
Rainfall	4.73 inches	3.78 inches
Maximum temperature	86° F.	91° F.
Minimum temperature	35° F.	44° F.
Collecting Periods	.....	.....
JULY & AUGUST		
Rainfall	3.47 inches	1.25 inches

The amount of rainfall previous to the collecting seasons totals approximately the same for both years. However, in May 1917 there was an interval of 16 days of dry weather as compared to a shower every two or three days in 1919. During the time collections were being made in 1917, 3.47 inches of rainfall was recorded as compared to 1.25 inches during the collecting period of 1919. Most of the rain that fell during the 1919 collecting season came near the close of the season in one or two heavy showers. The small amount of rainfall during the collecting season of 1919 might account for the low percentage of infection with some forms as compared with the season of 1917. For instance, hosts taken at Reese's Bog during 1919 contained no Diplodiscus, and no Cephalogonimus were found in any hosts examined that year. The percentage in the majority of instances runs lower for 1919 than for 1917.

Drought seriously affected the food plants of many insects in some habitats during 1919, and if insects enter into the life history of some parasitic forms, then this might account for the absence of adult parasites. The temperature of this region does not vary enough from season to season to be reckoned as an important factor. Erosion, weathering, and winds affecting the habitats are, no doubt, slight factors but some that cannot be entirely ignored.

The writer takes this opportunity to express his appreciation to Dr. George R. LaRue, under whose supervision this work was done, for his encouragement and helpful criticism; and to Dr. W. W. Cort and Dr. M. M. Metcalf for their aid in the identification of certain species.

Summary

1. A study was made of the distribution of the parasites of frogs in the Douglas Lake Region, Michigan.

2. Two hundred eight hosts from eleven habitats near Douglas Lake were examined during the months of July and August of the years 1917 and 1919.

3. Three species of hosts were examined, namely, *Rana pipiens*, *Rana clamitans*, and *Rana cantabrigensis*.

4. Ten species parasitic in frogs (excluding the nematodes) were found. Six species was the highest number found in any individual host.

5. *Octomitus*, *Opalina*, and *Gorgoderina* were present at all stations. Fourteen individuals of the genus *Gorgoderina* were taken from one host. *Diplodiscus* was not taken from all habitats. *Pneumoneces medioplexus* seems to be evenly distributed in habitats studied. As a rule infestations by this species run high, instances of 70 and 72 from one frog being recorded. But very few hosts contained *Pneumoneces similiplexus*, *Cephalogonimus americanus*, *Clinostomum attenuatum*, and Proteocephalidae.

6. The light infestation of *Rana clamitans* with Opalina is remarkable compared to the heavy infestations of *Rana pipiens*.

7. All stages in the life histories of many of the flukes are not known. Many cercariae are present in a given locality. By a study of both adult forms and cercariae of a given station these stages in life histories may be worked out. If an adult does not happen to be present, then the cercariae found there, in all probability, are not of that species. Such information may prove valuable to any one making a study of the cercariae of any given area or locality. Doubtless the cercariae found at Sedge Point are not a stage in the life history of *Diplodiscus temperatus* as no adults seemed to be present at that station.

8. No Acanthocephali were found in any hosts examined.

9. No *Diplodiscus*, *Pneumoneces medioplexus*, and *Cephalogonimus* were found in *Rana clamitans*, and the infestation of this species with *Pneumoneces similiplexus* and Proteocephalidae is comparatively low.

10. Males and females seem to be infested to about the same degree.

11. The food of the hosts, consisting chiefly of insects, the abundance of which is largely dependent upon rainfall and vegetation, appears to be a factor affecting the presence or absence of certain parasites. This might account for the absence of certain species at the Carp Creek habitat in 1919.

12. The amount and time of rainfall appears to affect the presence of parasites at a given station. The striking examples were that *Diplodiscus* was not found to be present at Reese's Bog and that *Cephalogonimus* was not found at all in the year 1919.

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NEW RECORDS OF NORTH AMERICAN ENCHYTRAEIDAE*

By

Paul S. Welch

Our knowledge of the North American enchytraeid fauna is so meager that at the present time any definite record is of considerable importance. Various collections sent to the writer for identification have yielded specimens representing species previously known only from remote localities and it has, therefore, seemed desirable to make available those records which modify so strikingly present impressions as to the range of the species involved.

Marionina forbesae Smith and Welch

During the month of August, 1921, Mr. R. L. Mayhew collected sexually mature aquatic enchytraeids from a small pond near the shore of Burt Lake, Michigan. Preparations resulting from a preliminary examination were subsequently transmitted to the writer for identification. Three specimens in the form of serial sections and three as whole mounts, all prepared by Mr. Mayhew, have been the basis of the work, although alcoholic material was also available.

Identity.—These enchytraeids exhibit characters which agree exactly with those described for *Marionina forbesae* Smith and Welch (1913), except that (a) in the Michigan specimens the dorsal blood vessel arises in the posterior part of XIV or the anterior part of XV, instead of in XIII as in the type, and (b) that small scattering unicellular glands occur at the ectal opening and along the surface of the spermathecal duct, such glands being absent in the specimens from Illinois. The writer has not felt justified in regarding these differences as representing more than variations within the species.

Mr. Mayhew's records contain no mention of the definitely arranged superficial spots reported in the original description of the species. Since alcohol causes these spots to disappear their absence in the Michigan material may be due to the preservative.

Delphy (1919; 1921) holds that the distinction between *Marionina* and "*Pachydrilus*" is not valid. Smith and Welch (1913) had already noted the close similarity between the two genera. However, pending more extensive study, the writer has followed the older practice.

Previous record.—*Marionina forbesae* was originally described from five sexually mature specimens found in the bottom mud and settlings of the waterworks reservoir at Urbana, Illinois, in October and November, 1895. This constitutes the only previous record.

* Contribution from the University of Michigan Biological Station, and from the Zoological Laboratory of the University of Michigan.

Habitat.—The specimens on which this identification is based were collected from masses of algae growing upon partly submerged boards in a pond, known as Fontinalis Run, on the northeast shore of Burt Lake, Michigan, about three miles from the University of Michigan Biological Station. This pond is merely an expanded end of a stream surrounded by swamp conditions and opening into Burt Lake through a narrow, shallow passage. A profusion of invertebrate animals and aquatic plants, large quantities of decaying organic matter on the bottom, floating wood bearing masses of algae, very slow current, protection from surface disturbances and complete absence of artificial influences are outstanding features of this habitat.

Fridericia bulbosa (Rosa)

1. A collection made at Mound City, Kansas, July 9, 1914, contained sexually mature enchytraeids six of which were studied in detail and found to be typical forms of *Fridericia bulbosa*. These worms were collected from decaying roots of alfalfa, under conditions which indicated an indigenous species.

2. A collection made at Emporia, Kansas, on June 22, 1921, and sent to the writer by Professor R. C. Smith, Kansas State Agricultural College, contained sexually mature specimens of *Fridericia bulbosa*. As in the previous case, these worms were found in connection with the dead or dying roots of alfalfa and apparently represent an indigenous species.

3. Sexually mature material of an enchytraeid was found by an inspector of the Federal Horticultural Board, on March 17, 1917, in the soil around the roots of citrus plants growing in the plant quarantine greenhouse at Washington, D.C. The specimens are clearly *Fridericia bulbosa*. The original source of these worms is uncertain. Mr. E. R. Sasscer of the Federal Horticultural Board, who sent the specimens, stated that the plants were originally received from the plant introduction garden of the Office of Foreign Seed and Plant Introduction at Yarrow, Maryland and "in all probability, the soil used comes from that locality." Considering the ease with which these enchytraeids are transported in soil about the roots of plants the original stock may have been imported from a foreign locality. However, the finding of representatives of the same species in central United States under conditions not indicative of foreign importation suggests the possibility that the Maryland material may also be indigenous.

Penial bulb.—Specimens from the three above mentioned collections all agree in the structure of the penial bulb. This organ is of the lumbricillid type in all respects. The body of the bulb is composed of cells of one kind only, their nucleated portions being near the periphery. A very few nuclei appear irregularly in the central region. Stephenson (1911,

p. 63; Pl. II, fig. 17) mentions and figures the penial bulb in specimens from the littoral region of the Clyde and as nearly as can be judged from the very brief description and the small figure there is complete agreement with the American specimens.

Chylus cells.—In the Maryland material the chylus cells occur in XIV-XVI, while in specimens from both of the Kansas collections the chylus cells begin in XIII and appear to end in XV.

Previous North American Records.—Moore (1895, pp. 343-344) described a new species under the name *Fridericia parva* from material collected in the vicinity of Philadelphia, Pa. Michaelsen (1900, p. 96) regarded *F. parva* as a synomym of *F. bulbosa* and more recent studies indicate the correctness of this view. If, then, the Philadelphia material be regarded as *F. bulbosa*, it constitutes the first and only North American record in the literature.

Fridericia agilis Smith

Through the courtesy of the Illinois Natural History Survey the writer had the opportunity to study some enchytraeids collected in the Sangamon River bottoms near Kilburn, Illinois. These worms were found about the roots of winter killed wheat in dark soil having a rather high moisture content and were reported as occurring in considerable abundance in region where the collections were made. About forty specimens were collected on April 5, 1912, of which thirty were sexually mature.

The color of the living specimens was, in many cases, nearly white with a slight tinge of flesh color. Some individuals were, however, distinctly yellowish throughout their entire length. They were very active and when disturbed showed vigorous writhing movements involving strong side to side motions. Serial sections and dissections showed that the specimens represent *Fridericia agilis* Smith. Aside from certain variations in size they agree with the original description of the species in every respect. The original description gives the variation in length of well-extended living specimens as 25-30 mm. The Kilburn material showed a range of from 20 to 29 mm., with an average of 24 mm. However, these measurements were made on alcoholic material and possibly show a lower range because of a certain amount of contraction during the killing process. The original description gives the number of somites as 57-66, the average being 62, while the Kilburn material shows a range of 52-69, with an average of 57. The diameter of the body in the region of the clitellum is 0.61 mm.-0.75 mm., average 0.68 mm.

This is the first time that *F. agilis* has been taken since the original material was collected by Professor Frank Smith (1895) in the vicinity of Havana, Illinois.

Enchytraeus albidus Henle

On June 5, 1914, enchytraeids were found in connection with the roots of house plants at Houghton, Michigan. Professor R. H. Pettit, Michigan Agricultural College, sent to the writer sexually mature specimens which proved to be typical *Enchytraeus albidus Henle*, the first record of its occurrence in North America west of the Atlantic Coast. Previous North American records have been discussed by the writer (1917, p. 120-121) in an earlier paper. The meager data accompanying the specimens give no information as to their original source. Their occurrence in connection with the roots of house plants makes it uncertain whether they have been transported thither with potted plants or are present in the native soil.

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PRIMITIVE MICROSCOPES AND SOME EARLY OBSERVATIONS[1]

By

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The question "Who first constructed the microscope?" is not one of major importance. The story is somewhat involved. However, the period in which magnifying glasses were brought into general use for the study of nature is quite well established. This was near the close of the sixteenth and in the first part of the seventeenth century. Primitive microscopes and pioneer observations with these instruments are of unusual interest, because they represent the tools employed and the beginnings of a new kind of scientific knowledge. Nothing of this kind comes down to us from antiquity. We should like to believe that Aristotle, the Alexandrines, and Galen had means of increasing their natural vision, but no such evidence exists. The unexpected discovery of so many appliances of antiquity has placed the modern mind in a receptive condition to all sorts of suggestions regarding the equipment of the ancients.

A lens-shaped rock crystal, discovered by Layard in the ruins of the palace at Nineveh, has been heralded as a quartz lens of great antiquity. This antique ornament or jewel, dating from 721-705 B.C., is now in the British Museum, and, as Myall, Charles Singer, and others have pointed out, its surface is not ground smooth but is cut into small facets, which disperse the light, so that it cannot act as a lens. Moreover, this piece of quartz is not clear but is clouded by dark bands. "From a number of sites of classical antiquity crystal balls have been recovered and these may or may not have been used as burning-glasses. The point is doubtful, but it is certain that they are not lenses in the usual sense of the word." (Singer.)

The fragmentary and usually dubious references to magnifications by ancient writers are not satisfying. The most often quoted statement is from Seneca's Natural Questions (63 A.D.), in which he says: "I may now add that every object much exceeds its natural size when seen through water. Letters however small and dim are comparatively large when seen through a glass globe filled with water." In this connection Seneca is attempting to explain why the rainbow appears so large, and the rest of the text shows that he is merely sustaining his hypothesis that objects seen through water appear enlarged; his mind is not directly concerned with the magnifying properties of transparent curved objects.

Passing over the story of the use of lenses by Alhazen in the eleventh, and Roger Bacon in the thirteenth century, we come to the last part of

[1] Address of the Retiring Chairman of the Section of the History of Science, American Association for the Advancement of Science, Boston, Dec. 27, 1922.

the sixteenth century where we can trace more directly the manufacture and the use of magnifying lenses. There are various claimants for priority, but it is not clear to whom the credit belongs. There were a number of spectacle makers at that time in the Netherlands, Italy, Germany, etc., and it would seem that combinations of lenses inserted in the ends of tubes were happened upon independently by different parties. In these early days the development of telescopes and of compound microscopes runs a parallel course. The simple microscope, consisting of a single lens, appears to have been used before lenses in combination, but both kinds were often employed by the same observer. After recognizing the Englishman, Digges, (in 1571), and the Hollander, Zacharias (called Jensen), about 1590, as prominent among the earliest inventors, we venture to say that to determine who actually was first is a small matter compared with who first made the instrument the common property of science. For this honor, perhaps, Galileo has the best claim. He was, says Charles Singer, the "effective" inventor of the telescope and the compound microscope. About 1608 he made his first telescope (soon followed by enlarged and improved forms); and with this combination of lenses he not only made observations on the celestial bodies, but, also, in 1609, published microscopical observations on minute objects.

We know, as a matter of fact, that single lenses (and lenses in combination) had been used earlier and that the use of magnifying glasses for scientific purposes came about gradually. A considerable number of early works exist of insects, spiders, worms, etc., some of them showing enlargements. For illustration, George Hoefnagel published in 1592 a set of fifty plates of insects engraved on copper. The pictures had been exquisitely drawn by his son, Jacob, at the age of seventeen, and some of them unmistakably indicate the use of magnifying glasses. So far as known the pictures of Hoefnagel are the earliest printed figures of magnified objects. There is reason to believe, however, that the naturalist, Mouffet, had made an earlier use of magnifying lenses. His "Theater of Insects" ("Insectorum sive Animalium Minimorum Theatrum") was prepared in manuscript as early as 1590 but was not published until 1634. Some of the illustrations in this book show magnifications.

In the complicated question regarding the invention of microscopes, involving conflicting accounts, Charles Singer offers some deductions as follows: 1. The invention of the microscope probably preceded that of the telescope. 2. The invention of the microscope was the work of Zacharias Jensen, after 1591 and before 1608. It was perhaps formed of two convex lenses. 3. This invention was followed by that of the telescope, about 1608, by Lippershey and Metius. Its military application drew attention to it. 4. The first telescope was of the Galilean type concave eye-piece and convex objective. Galileo, however, made both the telescope

and the microscope the property of science and was the *effective* discoverer of both. His instrument was improved by Kepler in 1611. The priority of effective demonstration of the telescope rests with Galileo and of the publication of a mathematical analysis with Kepler.

There is plenty of documentary evidence from writings in English, French, German, Dutch, and Italian to establish the fact that the use of the simple microscope was common in the first half of the seventeenth century. By the time of Harvey evidently magnifying glasses were no novelty. In his "De Motu Cordis et Sanguinis" (published 1628), he speaks in a matter of fact way in two places of his use of magnifying glasses.

A few years later we have the earliest printed pictures of microscopes, when, in 1637, Descartes published his "Dioptrique" as an appendix to his well-known "Discourse on Method" and supplied two pictures with descriptions of microscopes. Fig. 1 shows Descartes' picture of a simple

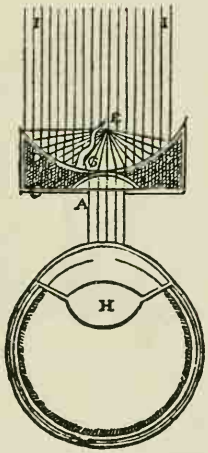

FIG. 1. Earliest known printed picture of the simple microscope. Descartes, 1637. (After Petri.)

lens provided with a means of illuminating the object to be examined. H represents the eye, in front of which, at A, is a plano-convex lens inserted in a blackened frame; behind the lens is a parabolic mirror with a transparent central area, through which the object can be viewed; the parallel rays of light from the mirror coming to a focus at the point, E. The object to be examined is attached to an object-holder, G, at the point of greatest illumination.

In addition to the foregoing, Descartes published a sketch of a huge clumsy apparatus designated an "ideal microscope." As shown in Fig. 2,

this has a sliding tube carrying a combination of lenses; the lens near the eye being plano-concave, and that at the far end of the tube (R) plano-convex. For illuminating the object, there was a concave mirror, similar to that of his simple microscope, and also a plano-convex lens placed in the pathway of light and giving a strong illumination at the point Z. Descartes says that the single lens may be replaced with one having two lenses combined. It is evident from these pictures and descriptions of Descartes that, in 1637, he had represented both the simple and the compound microscope. The large, unwieldy apparatus was later called perhaps in derision, a "megaloscope," but so far as known it remained as a theoretical representation and was never manufactured.

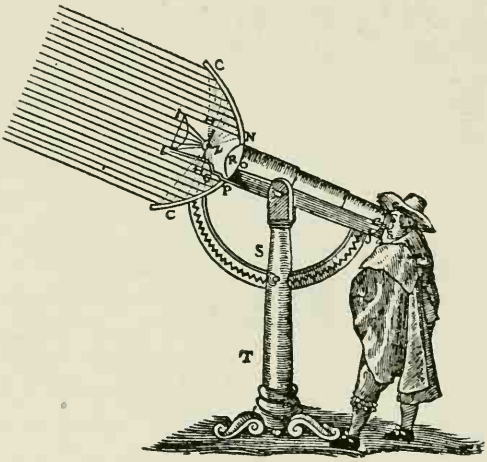

Fig. 2. Descartes' representation of an "ideal microscope," 1637. (Petri.)

The pictures of Hoefnagel and Mouffet, referred to a moment ago, were merely enlargements of objects visible to the unaided eye, but in the writings of Athanasius Kircher we have the first authenticated notices of microscopically minute living organisms. In his "Ars Magna Lucis et Umbrae," published in 1646, he describes a sphero-hyperbolic lens with which he made his first observations. Later he used an improved compound apparatus. Speaking of the different kinds of microscopes known in his time, Kircher says that some use two convex lenses; others use large glass globes filled with water and still others use a new and clever discovery of the smallest glass globules not larger than the smallest pearl. With the aid of lenses Kircher saw minute "worms" in all decaying substances, in milk, and in the blood of persons stricken with fever.

In 1658, in his "Scrutinium Pestis," Kircher gave a notable anticipation of the germ theory of disease. He described living "corpuscula" as occur-

ring in great numbers in the blood of plague stricken persons and stated that these micro-organisms were the source of contagion. Kircher did not see the organisms that produce bubonic plague—which were discovered a long time afterward—the structures which he saw were probably pus-cells and rouleaux of blood corpuscles, but he did ascribe contagion to living organisms (contagium animatum). More than one hundred years earlier "with remarkable clairvoyance," Fracastorius had attributed diseases to minute bodies or spores but he did not regard them as living organisms. Kircher's opinion was fortified by his actual observation of minute "vermicula" occurring in all putrifying substances and in the blood of the sick; his conclusion had some observational basis and his idea that infection is due to living organisms was a remarkable anticipation which has received merited attention in recent times. In following this idea of infection from living organisms, we note that a hundred years later, in 1762, Plenciz believed that there was a particular organism (seminarium) for each disease with a definite incubation period, but this noteworth example of prevision (together with others of similar import) was forgotten and the matter subject was revived only in the nineteenth century.

We now look with interest on the picture of Kircher's early microscopes. Fig. 3 from his "Ars Lucis et Umbrae" shows a short tube with a lens

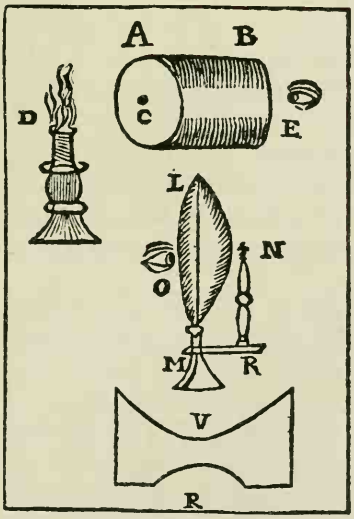

Fig. 3. Kircher's microscope, 1646. (Petri.)

at one end and a plain glass at the other. Another picture, Fig. 4, shows ornamentation of the tube. The object to be examined was placed against the flat glass and the lens near the eye was the magnifier. This is the prototype of the simple microscope. Because they were first used for

magnifying insects, these instruments came to be known as flea-glasses, and fly-glasses (vitrea pulicaria, vitrea muscaria, etc.). They were small tubes not thicker and longer than the thumb. In the last part of the seventeenth century they had quite a vogue as instruments of diversion, and documentary evidence shows that in 1679 microscopes with spherical lenses (microscopia globularia) were on sale in Paris.

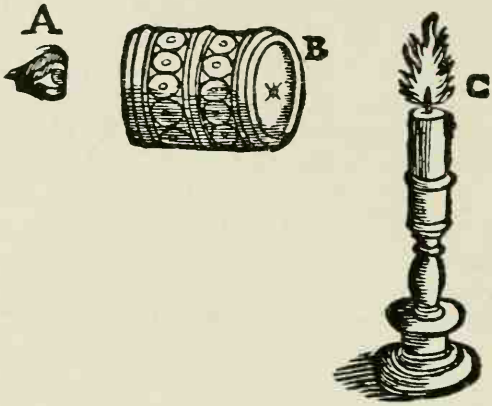

Fig. 4. An early "flea-glass" with ornamentation of the tube. Zahm, 1685.

In connection with Kircher, we should mention Schott, his colleague and fellow member of the society of Jesus. Kircher being occupied with another work besought his friend, Schott, to finish for him and publish a work on natural magic; this was done, and, in 1657, a year before Kircher's "Scrutinium Pestis" appeared, Schott published a sort of preliminary volume designated "Magia Optica" and giving credit to Kircher. The work was translated and printed in German, in 1671. I have had the use of this German edition through the courtesy of its owner, Dr. A. B. Luckhardt, of Chicago.

Fig. 5 is a photograph of the plate of microscopes in Schott's book. The size of these microscopes has been misconceived on account of the full-length human figure represented in connection with them and it has been generally overlooked that the dimensions of the instruments are mentioned in the text. Schott says of the picture marked 1 in the cut that the microscope is a small tube of wood or bone scarcely longer and thicker than a finger ("das kaum lenger und dicker ist als ein finger Glaich"). At the end near the eye it is provided with a small spherical glass not larger than the smallest pearl. The others also are described as relatively small. The dimensions of picture 4, the largest one represented, is given as having a tube a foot long and thicker than the thumb mounted perpendicularly on a small block three feet high. These instruments were not huge "megaloscopes" as represented in Descartes' "ideal miscroscope."

The presumption is that the artist inserted an entire human figure in place of the single eye commonly shown in many similar pictures.

In other sources of the nearby period we have an occasional mention of the size of the instruments employed. For illustration, Hooke's compound microscope (Fig. 11, about 1660), had a tube six or seven inches long, and a picture supposed to represent the microscope of the Italian, Divini, shows an instrument provided with five lenses, the length of which, by different writers, has been estimated from one foot to sixteen and one-half inches. In connection with the introduction of the microscope as a tool of science there naturally comes the discovery of micro-organisms, both animals and plants, and also the minute structure of tissues, of organic and of mineral substances.

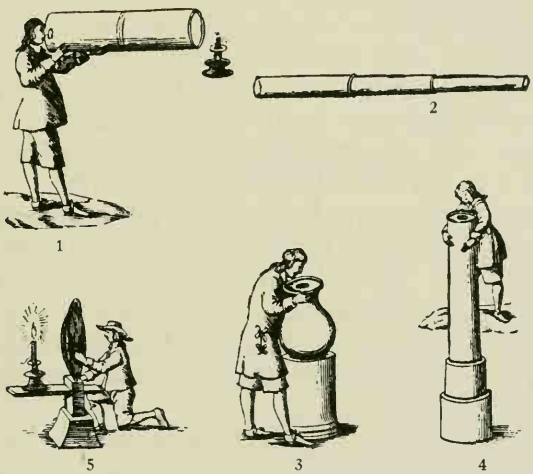

Fig. 5. Microscopes from Schott's Magia Optica, 1657. (Petri.)

The first to devote a long life to studies with the microscope, and to make a large number of observations—sometimes illustrated with sketches—was the Dutch observer, Antony van Leeuwenhoek of Delft. Through his multitudinous observations, published chiefly in the Transactions of the Royal Society of London and extending over a period of forty years, he made the microscopical world known to a wide circle. We may cluster about the name of Leeuwenhoek the story of early microscopical observations—remembering that there were other men who took part in the development of this kind of knowledge. In particular, Malpighi, the Italian, earlier in the field than Leeuwenhoek, extended his observations to the embryology of animals, to the minute structure of plants, to circulation of the blood in the transparent lungs of the frog (1660), etc., and Swammerdam, who used lenses extensively in investigating the structure of insects.

Leeuwenhoek made his observations with small microscopes of his own contrivance. Although he made several hundred of these instruments for his own use, he was not, as represented in Dr. Carpenter's article in the ninth edition of the Encyclopaedia Britannica, an optician, nor a manufacturer of lenses for the market. Time does not permit now to demonstrate this point.

Twenty-two years before his death, Leeuwenhoek designated twenty-six of his microscopes to go to the Royal Society after his death. His communication to the Royal Society was dated Aug. 2, 1701, and since it throws light on the extent to which he prepared his own instruments, it is worth quoting: "I have (says Leeuwenhoek) a small black cabinet, lacker'd and gilded, which has five little drawers in it, wherein are contained thirteen long and square tin boxes, covered with black leather. In each of these boxes are two ground microscopes, in all six and twenty; which I did grind myself, and set in silver; and most of the silver was what I had extracted from minerals, and separated from the gold that was mixed with it; and an account of each glass goes along with them.

"This cabinet, with the aforesaid microscopes, (which I shall make use of as long as I live), I have directed my only daughter to send to your Honors, as soon as I am dead, as a mark of my gratitude, and acknowledgment of the great honor which I have received from the Royal Society."

Baker, in his work 'The Microscope Made Easy' (1742), mentions having had these instruments away from the rooms of the Society for examination. He described them and figured some of them, but soon after they were lost sight of, and, unfortunately, these hierlooms to science have never been recovered.

Inasmuch as Baker had these microscopes under observation his testimony as to the shape of the lenses is important. He says: "Several writers represent the glasses Mr. Leeuwenhoek made use of in his Microscopes to be little globules, or spheres of glass; which mistake most probably arises from their undertaking to describe what they had never seen; for, at the time I am writing this, the cabinet of Microscopes left by that famous man, at his death, to the Royal Society as a Legacy is standing upon my table; and I can assure the world that every one of the twenty-six Microscopes, contained therein, is a double convex lens, and not a sphere or globule."

Leeuwenhoek gave descriptions and some drawings of his microscopes, and those in existence have been described and figured by different writers, so that we have a very good idea of his working equipment. He preferred the single lens, with a small glass of marked curvature, giving a small field but clearer definition than the compound microscope of Hooke. He made different microscopes to suit his purposes, having a range of magnification from 40 to 270 diameters.

One of Leeuwenhoek's originals exists at the University of Utrecht, and at my request Professor H. F. Nierstrasz photographed this instrument natural size. Three views of his photographs are shown in Fig. 6.

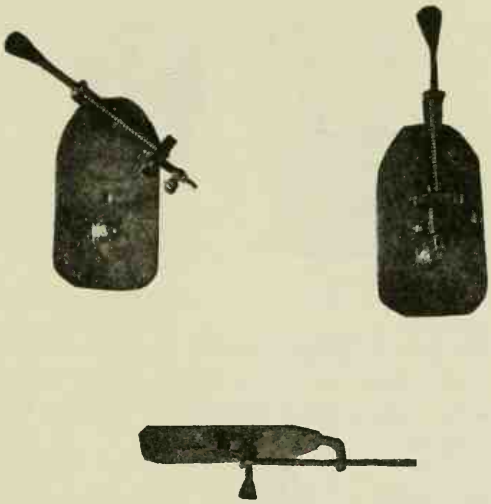

Fig. 6. The Leeuwenhoek microscope in the University of Utrecht. Photographed by Professor Nierstrasz.

The instrument has two small copper plates, perforated by an orifice in which the small, nearly spherical lens is inserted. In the original, the copper plates measure one inch broad and a little short of two inches long. The object-holder is represented in the lower right-hand figure as thrown to one side. By a vertical screw the object could be elevated or lowered, and by a transverse screw it could be brought near or removed farther from the lens and thus be brought into focus.

In use, the instrument was held close before the eye (Fig. 7) against the light, and the object was viewed by transmitted light.

Fig. 7. To show how the Leeuwenhoek microscope was held. (Petri.)

In some instances, however, the microscope was provided with a concave reflector (Fig. 8) similar to that used by Descartes, to illuminate the object by reflected light.

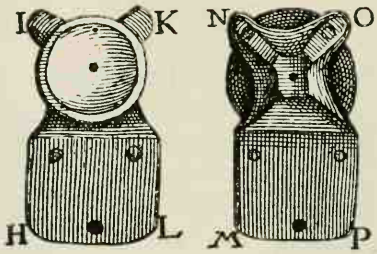

Fig. 8. A Leeuwenhoek microscope provided with a concave reflector. (Petri.)

Fig. 9 shows the way in which the microscope was arranged by Leeuwenhoek to examine the circulation of blood in the transparent tail of a small fish or tadpole. The animal was placed in water in a slender glass

Fig. 9. Leeuwenhoek's arrangement for examining circulation of the blood.

tube, and the latter was held in a metallic frame to which a plate (marked D) was joined, carrying the magnifying glass. The latter is indicated in the circle above the letter D, near the tail-fin of the animal. The eye of the observer was applied close to the lens which was brought into position and adjusted by means of screws.

Of the many discoveries of Leeuwenhoek, we can give only one example. This will be his observation of the bacteria; since it is the earliest account of bacteria accompanied with sketches, it is of especial interest. The

discovery of these minute forms was a feat of trained observation, and it is remarkable that Leeuwenhoek, with his primitive equipment, was able to see them and to describe them so clearly. One of his letters of 1681 indicates that he had seen bacteria at that date, but his formal description of them came in 1683. There can be no doubt from his sketches and descriptions that he saw the chief forms of bacteria—round, rod-shaped and spiral forms.

His first observations on bacteria were communicated to The Royal Society of London, in a letter dated Sept. 17 (not 14), 1683, and published in the Philosophical Transactions for the year, 1684.

A photograph of the cut published with his observations is shown in Fig. 10. It may be remarked in passing that the reproduction of the cut by Löffler, Petri, and others, is not quite facsimile and their quotations

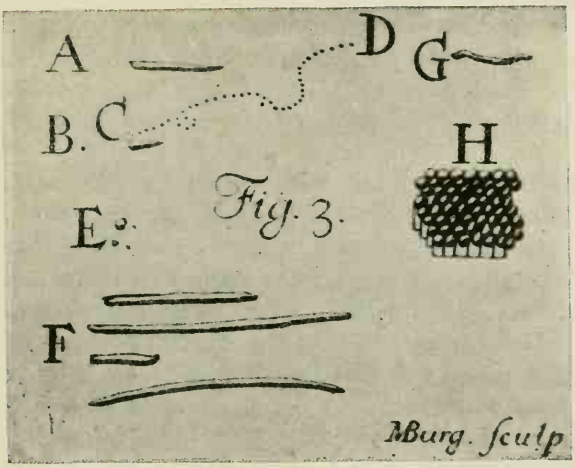

Fig. 10. Photograph of the original plate of bacteria as seen by Leeuwenhoek in 1683. (After Charles Singer, from the Philosophical Transactions, 1684.)

do not correspond verbally with the text in the Philosophical Transactions. A few lines from the original publication in the Philosophical Transactions shows the objective quality of Leeuwenhoek's descriptions:

"Tho my teeth are kept usually very clean, nevertheless when I view them with a Magnifying Glass, I find growing between them a little white matter as thick as wetted flour: in this substance tho I could not perceive any motion, I judged there might probably be living creatures.

"I therefore took some of this flour and mixt it either with pure rain water wherein were no animals; or else with some of my Spittle (having no Air bubbles to cause a motion in it) and then to my great surprize perceived that the aforesaid matter contained very many small living

Animals, which moved themselves very extravagantly. The biggest sort had the shape of A (see the cut). Their motion was strong and nimble, and they darted themselves thro the water or spittle, as a Jack or Pike does thro the water. These were generally not many in number. The 2d. sort had the shape of B. These spun about like a top, and took a course sometimes on one side, as shown at C and D. They were more in number than the first. In the 3d. sort I could not well distinguish the Figure, for sometimes it seem'd to be an Oval, and other times a Circle. These were so small that they seem'd no bigger than E. and therewithal so swift, that I can compare them to nothing better than a swarm of Flies or Gnats, flying and turning among one another in a small space. Of this sort I believe there might be many thousands in a quantity of water no bigger than a sand tho the flower were but the 9th. part of the water or spittle containing them."

"Besides these Animals there were a great quantity of streaks or threads of different lengths, but like thickness, lying confusedly together, some bent, and some straight as at F. These had no motion or life in them, for I well observed them, having formerly seen live-Animals in water of the same figure."

Leeuwenhoek extended his observations to others: two women; a child of 8 years old; the spittle of "an old man that had lived soberly; and another old man who was a good fellow." The "meal" between the teeth of the old men "had a great many living Creatures, swimming nimbler than I had hitherto seen. The biggest sort were numerous, and as they moved, bent themselves like G. The other sorts of Animals were in great numbers insomuch that tho the meal were little, yet the water that it was mixt with seem'd to be all alive, there were also the long threads above mentioned."

The figure marked "H" has very generally perplexed writers, and has been designated by some as a representation of those round bacteria which occur in packets of cubes (sarcinae), but later in the same paper, Leeuwenhoek says that "H" represents scales of the outer skin (cuticula).

It is worthy of note that bacteria were pictured before protozoa (which had been discovered by Leeuwenhoek in 1675), and if we except the poor picture of a shelled-protozoan, (Rotalia), by Robert Hooke, in 1665, they were, I believe, the first micro-organisms to be illustrated in printed pictures.

Fig. 11 is a picture of Robert Hooke's compound microscope, made about 1660, and constituting the frontispiece of his Micrographia, which, in 1665, was published as the first book devoted expressly to microscopical observations. This shows the form to which the compound microscope had attained in the last part of the seventeenth century. Space does not permit us to follow its further development through the eighteenth and

nineteenth centuries. Hooke's Micrographia gave a real impetus to observations with the microscope, especially in England. Among others, Neimiah Grew, the fellow countryman of Hooke, was stimulated by its publication to carry on his extensive observations on the microscopic structure of plants.

The psychological influence of the use of the microscope was very great. By sharpening attention and directing it towards definite points, the powers of mental application were improved and impressions received through the sense of sight were made more exact. Now, perception through

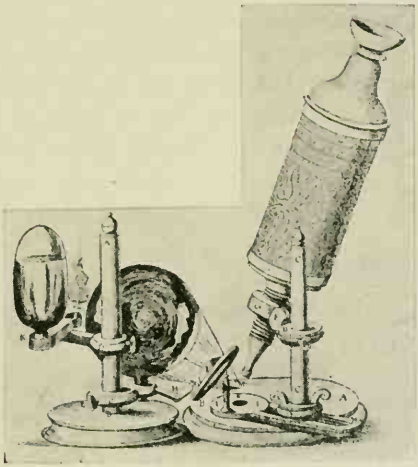

Fig. 11. Hooke's compound microscope (about 1660). From his Micrographia, after Carpenter.

trained senses is the foundation of all scientific knowledge, and, as a matter of fact, we find the early workers with the microscope, Robert Hooke, Malpighi, Grew, and Leeuwenhoek, seeing nature more scientifically and exactly than their predecessors. As Sachs remarks in his History of Botany: "Preception by the use of the optic nerve had to be accompanied by conscious and intensive reflection, in order to make the object, which is observed only in part by the magnifying glass, clear to the mental eye in all the relation of the parts to one another and to the whole. Thus the eye armed with the microscope became itself a scientific instrument, which no longer hurried lightly over the object, but was subjected to severe discipline by the mind of the observer and kept to methodical work." Although there was started a period of more incisive observation, the early microscopes were very imperfect and it was not until their improvement in the first third of the nineteenth century that the full effect of their use was realized.

AN ILLUMINATING DEVICE FOR MICROSCOPES

By
William A. Beck
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Introduction

Much attention has been given by the builders of microscopes to the selection of the correct combination of objectives and oculars for the study of various preparations. The importance of proper illumination has also been appreciated and much thought and care has been expended upon this phase of microscopic methods. There are difficulties presented, however, because of the very nature of things, so that much remains to be desired in modes of illumination for certain fields of investigation. The device which I am bringing before the profession is an effort to improve the illumination.

Modes of Illumination

Bright Field Illumination.

About 95% of the study of microscopic objects is done in a bright field. The entire field is lighted and the objects to be studied appear as colored objects, with the different tones severely bounded, each for itself being homogeneous within its own bounds. In extreme cases the objects stand out as silhouettes in their bright field. The very nature of the microscope has predetermined the development of this mode of illumination because of the relatively short working distance of the objectives. Any other mode was too limited in its application, particularly during times when an intense and concentrated source of artificial illumination was impossible.

The bright field illumination is obtained by allowing the light from the sun or some artificial source to fall directly or after diffusion, upon the mirror of the substage, whence it is directed, with or without the aid of a condenser, upon the subject under study, which must naturally be transparent or translucent. The light, thus directed, may be transmitted axially or obliquely or may be so thoroughly diffused by secondary reflection, that the illumination no longer has the character of being directed.

Dark Field Illumination.

In contrast with this is Dark Field Microscopy. Ordinarily the field is actually very dark and the object is bright or, in some cases at least, the light is deflected into the objective and gives the impression of a white object on a dark background. In other cases the background is not actually dark or of even tone, in fact it may be brighter than some tones of the image, but since this is due to secondary reflection under studied con-

ditions, so as to bring the subject into better relief by the same methods, we might well consider this mode under the heading of Dark Field Microscopy.

There are two principal cases. First when the objects are self-luminous i.e., phosphorescent, or when, by illumination with ultra-violet light, they become phosphorescent. Secondly, when the objects themselves do not emit light but which reflect or deflect the light, reaching them from some outside source, passes into the microscope. Different opaque objects having different reflecting powers, will under these conditions, produce widely different tones in the images produced. We might do well to borrow a term from Astronomy in describing them, where *albedo* is taken to mean the ratio of the quantity of light reflected to the quantity of light received.

If one looks into the sky and notes the stars against the dark vault, he has a good illustration of the first case, i. e., of darkground illumination. If he regards the planets he has an illustration of the second case.

My device pertains chiefly to the domain of Dark Field Microscopy and in particular to the Second Case.

History of Illumination of Opaque Objects

For progress in any field, the first step must always be the investigation of the principles involved. Later we find that development of knowledge in other fields are helpful in making proper application of these principles, in the attainment of a given end. The proper illumination of opaque objects under microscopic examination is no exception.

Early investigators appreciated that the principle of contrast was of the highest importance in rendering objects visible. The application of convex lenses for magnifying the objects was made by Roger Bacon as early as 1266. In 1610 Kepler devised the compound microscope with convex objective and convex ocular from which, in time, evolved the modern form. In this evolution, condensers, for lighting the objects, played a prominent part. In 1637 Descartes used a large parabolic mirror to direct sunlight upon the object. This mode of lighting showed the object more or less bright, on a dark background. His unwieldy apparatus gave way to more convenient microscopes and condensers, as progress was made in the various domains of science. It is interesting to note, however, how men have struggled down to the present time for a better application of a principle, recognized at an early period.

Lister appreciated the fundamental difference between Bright Field Microscopy and Dark Field Microscopy in 1830. It was not until Zeiss in 1904 and Leitz in 1905 made practical applications, which were later discarded for more effective devices, so that the object is illuminated, by beams of light, in such a direction with reference to the axis of the objective, that none of them can enter the objective directly and the light, going

into the microscope, comes only from the objects themselves, so that they appear self-luminous on a dark ground.

When the light is directed from below upon the object we do not expect to obtain a clear image of the upper surface of the object but are more concerned about discovering the existence of such objects and note their movements etc. The application of this principle has led to the wonderful developments in Ultra Microscopy.

If the light is directed upon the object from above and the object is over a non-reflecting background, the object will appear bright in a dark field.

From the time of Descartes, many efforts have been made to improve the mode of illumination from above. Reflectors were used to direct the light from the side onto the object, or again a "Bull's Eye" Condenser concentrated the light, from a specific direction, onto the object.

The Lieberkuhn reflector which was devised in 1740 is not unlike Descartes' device, in principle, where a parabolic mirror directs the light onto the object. Since 1850 two additional devices for illuminating from above, have come into use.

In 1852 Riddell suggested introducing the light from the side into the objective and reflecting it down upon the object. From this suggestion have resulted the various types of Vertical Illuminators. Some employ total reflection in a prism, to direct the light down through the objective, others a disk of glass and still others a mirror. A short time ago a new method was devised by Professor Alexander Silverman of the University of Pittsburgh. It consists of a circular electric filament lamp, which surrounds the objective and shines down upon the object.

The Illumination of an Object Under Study

Up to the present only directed illumination was employed in the microscopic study of opaque objects, to obtain a knowledge of their surface configuration.

The directed light was either oblique to the optic axis of the system of lenses or approximately parallel, i.e., either oblique or vertical with respect to the surface under examination. We might then simply use the terms oblique or vertical, to designate these modes of illumination. Both are to be considered as directed illumination, because we have a pencil of light from a definite direction.

Oblique Illumination.

Oblique illumination can be obtained either by means of a reflector attached to the objective or by directing rays from a radiant, lying above the plane of the surface of the object. When a radiant is employed, for example an arc lamp, a tungsarc, or a filament lamp, a condensing lens is

usually interposed, between the light and the object, in order to concentrate the light rays and to facilitate the proper placing of the beam. If we are dealing with a highly polished surface this mode of illumination has no value, because no rays can enter the objective according to the laws of regular reflection (Figure 1).

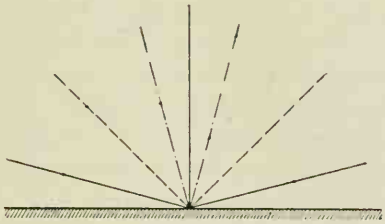


FIG. 1

Illustrating the law of regular reflection and showing how oblique illumination on a flat surface cannot direct the light into the microscope.

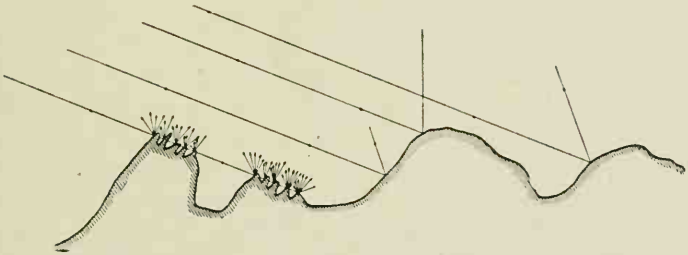


FIG. 2

Illustrating regular reflection on an irregular surface, showing how regions at the proper angle reflect an intense light into the tube of the microscope while others do not. Many points in close proximity may produce defraction patterns.

If the surface of the object illuminated is irregular or etched, the rays entering the objective from some points are very intense, while from others they may be entirely lacking and therefore do not express the relative albedo of the surface, nor give an impression of the plasticity of the object. This becomes clear at once from a study of figure 2. This figure also shows how certain regular markings in certain cases produce diffraction effects. Diffraction patterns make it difficult to interpret the true structure.

The greater the obliquity the greater the incident difficulties become, so that with high power objectives, where the free working distance is very small, this mode of illumination is entirely out of the question.

Illuminating from several sides tends to correct somewhat the difficulties referred to, in giving the body illuminated more of the character of a self luminous body, according to Huygens' impression, that each point illuminated becomes a new point source of disturbance. Then again dif-

fraction patterns are eliminated. Complete annular illumination was attempted in the paraboliod illuminator which was very popular at one time. For correct illumination, the curvature of a reflector placed around the objective would have to vary for each working distance. These were later almost entirely superseded by the vertical illuminators.

In the Silverman device, that has come into the field in relatively recent years, and which became popular immediately upon its appearance, the illuminant is attached directly to the objective. Here the illumination is about three fourths annular and consequently shows decided improvement over previous modes of illumination by the oblique method. There still remain these difficulties: the illumination is not completely annular. The angle of illumination cannot be varied at will. The intensity of illumination is limited. The light cannot be completely diffused. The light cannot be filtered at will. The life of the lamp is short. A considerable amount of undesirable heat is developed.

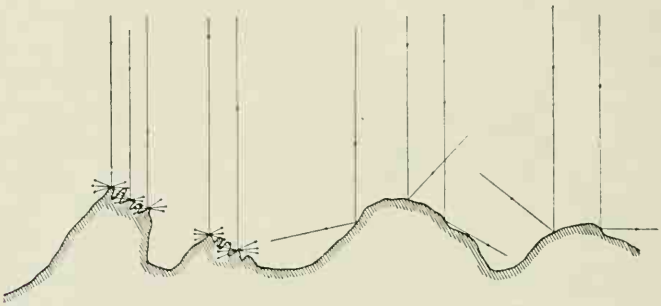


Fig. 3

Showing a rough surface under vertical illumination. Note that only a strictly horizontal surface can return the light directly into the tube of the microscope. Diffraction patterns cannot produce images in the field.

Vertical illumination is obtained by placing a reflecting surface in a mounted cell attached to the microscope, just above the objective. The reflector sends the illuminating beam of light through the objective, which acts as a condenser, concentrating the light rays into a bright spot of light, upon the surface of the object, at a point lying, approximately, in the optic axis of the microscope. From the surface of the object, the rays are reflected back through the objective and form the image of the object in the usual manner.

If the object to be studied is absolutely flat, it is evident that the rays reflected from the object might be considered as emitted by the object and these should form an image, that should give a correct impression of the *albedo* of the various parts of the field. Furthermore, if there

are sections, that otherwise would be inclined to form diffraction patterns, the law of regular reflection would require that all reflected and diffracted rays fail to enter the objective, not being parallel to the optic axis. (Figure 1, central beam. See also Figure 3 where points lie in close proximity.) There is absolutely no question that this type of illuminator has rendered, and is still rendering, immeasurable service to science. For certain work it may never be replaced.

When the object to be studied is not flat, the image immediately ceases to give the correct impression regarding albedo and the nature of the surface. The image needs interpretation. The investigator may be dealing with more or less highly polished surfaces and with areas, part of which, are polished, part rough and often studded with minute points. Sometimes cracks or cleavage planes may cross the field.

With ordinary etched surfaces, polished portions appear bright and etched surfaces less bright. The glare from the brighter surfaces, seeks to lessen the definition in the less bright fields. It is questionable if the detail of the mat surfaces are in any degree accurate. To demonstrate fissures, cleavage planes, depressions etc., it becomes necessary that the examination with the vertical illuminator be supplemented by oblique illumination and to study the direction of the shadows with respect to the radiant, remembering of course that in the image seen in the microscope, directions are completely reversed. The need of this study suggests a mode of oblique illumination, such that the light beam can be turned about the axis of the microscope, with least possible difficulty.

The Illumination of Macroscopic Objects

Since the principles for the correct illumination of objects, as we observe them in every day life, with the unaided eye, are the same as for microscopic study, we might do well to give a little attention to the phenomenal progress that has been made within the past decade by illuminating engineers in their proper sphere.

Prominent among the scientists who are working for better illumination to obtain correct values and at the same time to save the eyes, is Professor C. E. Ferree of Philadelphia. He has made a series of tests of the eye, under different kinds of illumination, daylight, indirect lighting, semi-direct lighting and exposed filament lighting.

He found that after three hours' work under day light, the eye lost practically nothing in seeing efficiency. Under indirect lighting the effect was almost the same; the eye was 91% efficient in seeing ability. Under exposed direct or semi-indirect lighting the loss in seeing efficiency was enormous; the remaining efficiency being only 25% with semi-direct and 14% with direct. Science has conclusively demonstrated, that if we would preserve our eyesight and obtain correct values of the tones in a

scene we must avoid, so far as possible, *glare* and *direct illumination*, because the eye cannot endure excessive light. Momentary blindness results from intense light because the entire retina or a portion of it becomes paralysed. The result is that we do not perceive objects within the range of vision that should be seen normally. The greater the glare of any spot in the field, the more intense the illumination must be before the other portions can be perceived, which increase however, serves to render the "spot" more glaring, thus defeating the purpose.

The Formation of an Image.

If in the neighborhood of a luminous point P, there are refracting and reflecting bodies having an arbitrary arrangement, then, in general, there passes through any point in space, one and only one ray of light, i.e., the direction which it takes from P to P′ is completely determined. Under given conditions, certain points may be formed, at which, two or more of the rays emitted by P intersect in a point P′ which is called the optical image of P. If we have many points in our object under study, the summation of all the images of these points will give a more or less accurate replica of the object, according as the numbers expressing rays, passing through the P′s do or do not bear the same ratios to each other, as the numbers expressing the rays actually emitted by the conjugate points in the object.

From what has been said of regular reflection, it is clear that we cannot form an adequate image of the reflecting object when it reflects regularly, but only of some other object that emits rays of light which come to us after reflection.

When the light falls in a definite direction on an unpolished surface it is reflected in various directions. The amount of light going in a given direction for the formation of an image depends upon the position of the image with respect to the position of the illuminant. It is clear then that the number of rays that combine to form an image, depends upon the particular position of the images, as well as upon the nature of the surface and the albedo of the object. This varying factor, of position, offers serious difficulty in the formation of a correct image. The thought naturally arises in the mind, that we attempt to illuminate equally from all sides in order that only the nature of the surface and the albedo shall determine the image.

Reflection of diffused light, produces not only correct form and tone, but is also selective, so that we maintain correct color value. The rich red petal of the geranium thus illuminated by white light, reflects diffusely in all directions only the red rays. In order to form correct images it follows from the above discussion that direct illumination must be avoided and diffused illumination employed, whenever possible.

Illuminating engineers corrugate reflectors and give the walls a rough finish rather than an even finish to avoid regular reflection. All their efforts are directed in distributing the light energy, like a fine spray, throughout the entire room. We are all familiar with the excellent results obtained by the modern modes of illumination.

Fig. 4

A study under Northern Sky illumination. Note the exactness of detail and the plasticity. The snow helps to diffuse and illuminate, so that the illumination is practically annular and diffuse. Copyright by John Mathews.

Photographers have long ago known that they obtain their best picture by the evenly distributed illumination from a northern sky Figure 4.

The commercial photographers recognize the importance of completely annular illumination and diffuse illumination and employ methods in which they obtain so called "cross-fire" of light, by which term they mean, that the light comes from all directions with almost equal intensity.

To illustrate the differences between obliquely directed illumination and vertical illumination and "cross-fire" illumination a bust of Schiller, which was painted with a dull even white, was photographed under the three conditions. Just as was expected marked differences were noted. The picture that was taken with the aid of oblique illumination, was so badly distorted by extreme contrasts and heavy shadows that it gave no impression whatever of Schiller's physiognomy. The vertical illumination produced a picture that was more even and lacked that extreme contrast, in fact was flat. The greatest difficulty with the picture was that it gave a false impression of the real appearance of the subject, because elevations

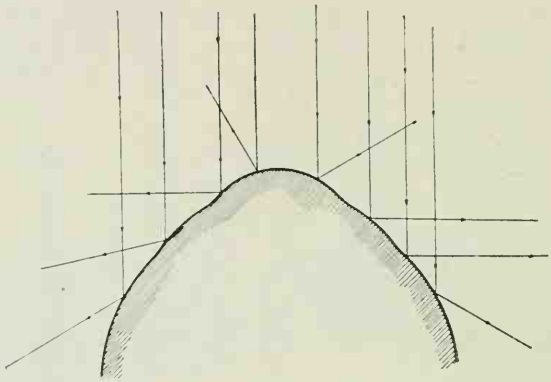


Fig. 5

This diagram illustrates how the light, from vertical illumination upon an elevation, fails to return to the observer or the instrument.

were dark and depressions were bright. This is just as it should be. A hill would cause the light to be dispersed while the valley would return too much of the light. This is clearly shown in figures 5 and 6. The third picture was of the nature of those ordinarily produced by portrait photographers, marked by correctness of form and surface variations and a remarkable plasticity.

The chief aim in view in designing the new type of illuminator was to obtain a diffuse or "cross-fire" illumination for microscopic objects, of a sufficient intensity to obtain in pictures, of the same relative value as in the third case of the photography of the bust.

The Illuminating Device

The device consists of a glass medium which completely surrounds the object under study (Figure 7). The light enters at the ground surface A, is totally reflected at the surface C, and is finally refracted at the surface D, and thus directed from all sides upon the object under study. The object rests upon a stage E_1, which is adjustable, so as to allow a variation

in illumination. The illuminator can be made of any convenient size. The one which I actually employ and which I recommend for most work, is so designed as to fit the sleeve on the substage, which ordinarily receives the Abbé condenser.

Any convenient light source may be employed. For ordinary study, a common filament lamp or the microscope lamp is satisfactory. With the gas-filled lamp, one can obtain greater intensity. Just as our modern illuminating engineers seek a great quantity of light very finely distributed, so also it is very advantageous to use an arc lamp in connection with the illuminator, distributing the intense light very evenly. The illuminator allows the microscopist to employ practically any intensity of light he desires.

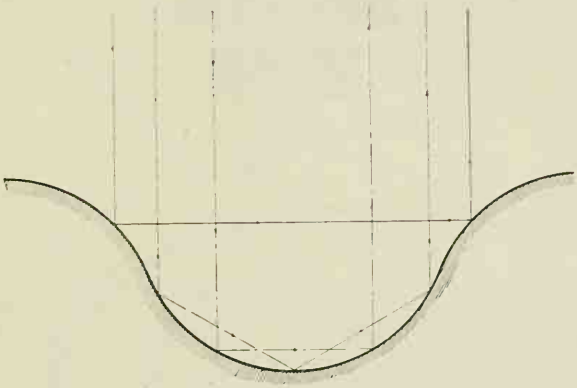


Fig. 6

This diagram illustrates how the valley returns too much of the light under vertical illumination and causes the values of the field to be reversed.

Plane parallel light is ordinarily to be recommended, which may be obtained by placing a lens in such a position that the light source is in the principal focus of the lens. By varying the position of the lens, one can readily vary the nature of the illumination and in a very interesting manner, simulate "spot-lighting." Direct or diffused sun-light can be employed as well as an artificial light source.

The light may be allowed to fall immediately upon the surface A_1 or to be reflected, from the mirror of the substage, onto that surface. If the mirror is employed one can "spot out" certain regions of the subject from one side or the other by simply turning the mirror. This makes it very easy to study the nature of a given object by means of shadows.

The angle which the side D_1 makes with the vertical, determines the deviation of the beam of light from the horizontal as it passes from C_1 to D_1. The deviation must be such that the entire field to be studied by the

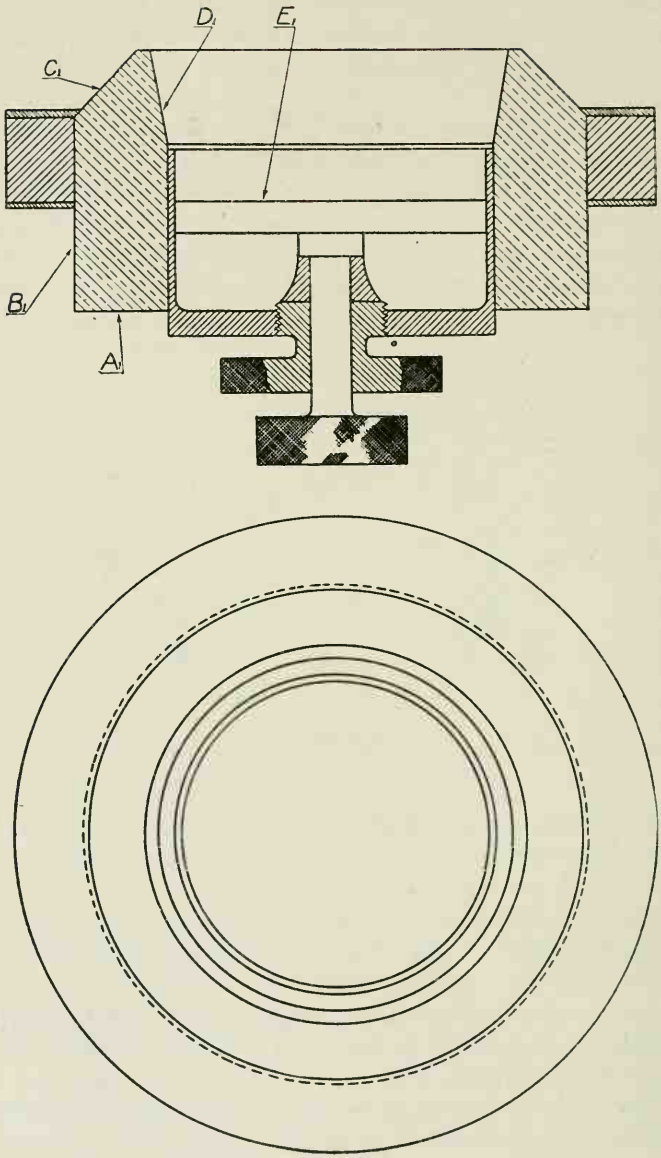


FIG. 7

Longitudinal section of the illuminating device and the plan of the same. B_1 is the glass medium mounted in a metal sleeve. The light enters the medium at A_1 and is totally reflected at the surface C_1 and thus directed to the surface D_1. From D_1 the light deviates from the horizontal direction and is directed to the stage E_1. The entire piece is interchangeable with the Abbé condenser.

determined objective, be sufficiently illuminated after the objective is in the position of the correct working distance.

The dispersion of the light depends upon the refractive angle of the device which is given by the angle which the face D_1 makes with the verti-

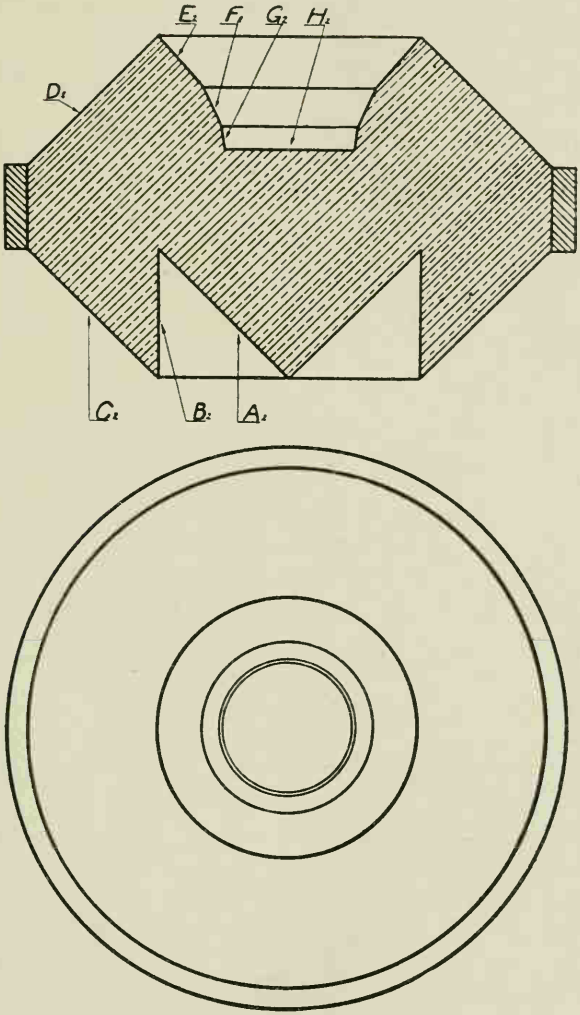


FIG. 8

Another form of the illuminator. This one is made to fit the large opening on the stage of the microscope when the entire mechanical stage is removed. Note the three different refracting angles where the light leaves the glass medium. The cone A_1 converts the solid cylinder of light into a hollow cylinder of light. This form can be made in one or more pieces. The light is received at A_2, transmitted at B_2, reflected at C_2, again reflected at D_2 and refracted at the surfaces E_2, F_2 and G_2, falling at different angles, upon the subject resting on H_2.

cal. This angle is always small and the angular dispersion in consequence is negligible, so that we have a condition of achromatism without special correction.

After a careful study of these values was made for the various objectives, it was found possible to have three refractive angles which would give highest efficiency for the various objectives that can be employed. If it is possible for the makers of objectives, to narrow down somewhat the diameter of the lower portion of high power objectives, there is no reason why this modification, should not make it possible to study any opaque object at the highest diameter of magnification, by this method of illumination.

To increase still further the intensity of illumination, a special design for the lower portion of the device was made to convert the solid cylinder of light, into a hollow cylinder of light, by means of a reflecting cone and totally reflecting surface.

These modifications gave rise to the form shown in figure 8. It is not necessary that the transparent medium be of one piece as the diagram indicates. Furthermore the arrangement for a variable inner stage is not shown. Finally the new device can be arranged in two units. One portion resembling the illuminator shown in figure 7, and the other representing the lower portion in figure 8, by means of which the solid cylinder of light is converted into a hollow cylinder of light.

The light can be diffused by placing a ground glass between the source and the illuminating device or by placing a diffusion medium around the object which receives the light from the surface D_1, (figure 7).

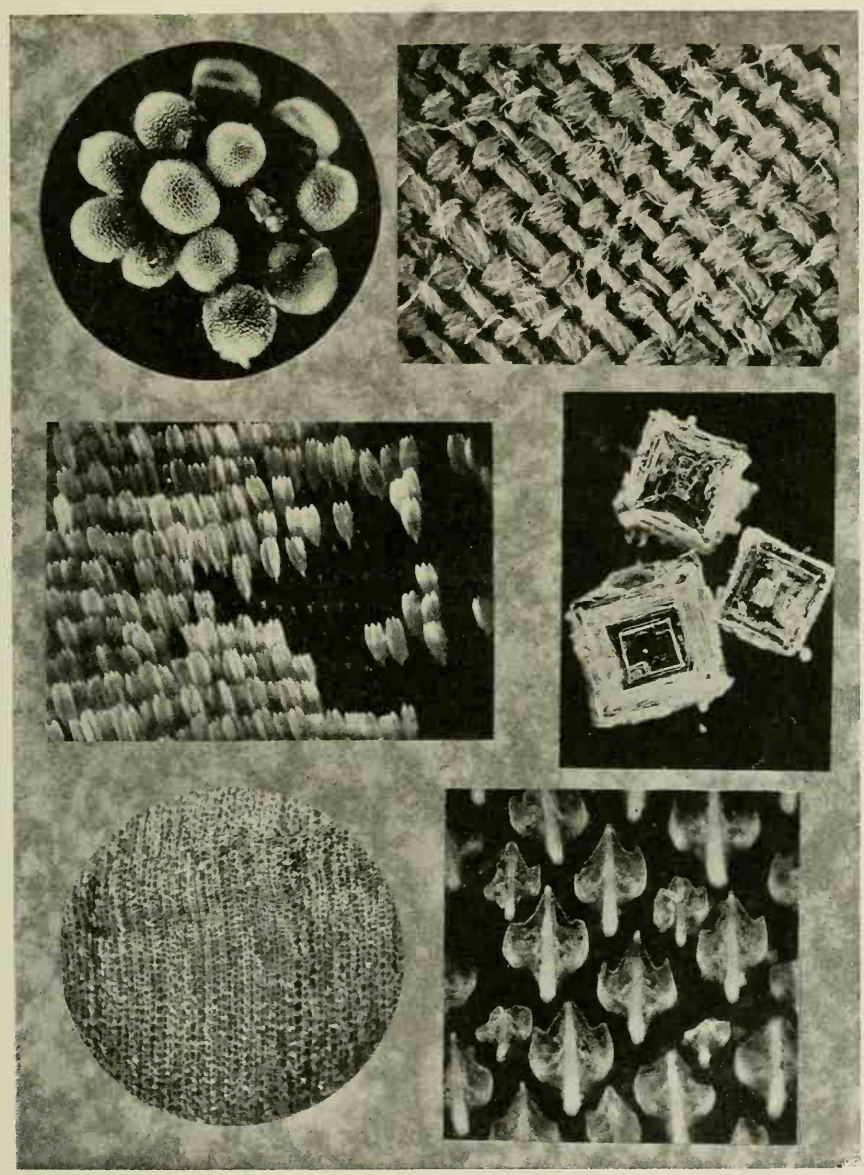
For certain work it is desirable to filter the light. Regular filters can be interposed between the source of light and the illuminator.

It is to be noted particularly that by this method we obtain completely annular illumination and that we can without difficulty obtain diffuse illumination resembling the illumination which photographers seek from the northern sky or such as is obtained by the devices employed by commercial photographers. If light shadows are desired one can very easily increase the illumination in a specific direction.

The device can be used simultaneously with the vertical illuminator. In an interesting experiment, both were set into position and first the light from the one and then light from the other was employed. It was interesting to note a complete reversal of the tones as explained above and indicated in figures 5 and 6.

The device was originally designed for the study of opaque objects but it can be used to advantage for the study of transparent objects by placing an uneven reflector, as a piece of filter paper, on the stage E and place the preparation, in the ordinary way, upon the regular stage of the microscope. The illumination thus obtained is remarkable for its softness, clearness and definition. One can study with less eye-strain when employing this method and the contrast is sharp, even though the light is not intense. This is just as it should be, according to the principles outlined above.

It is to be hoped that this method of illumination will be helpful in various fields of microscopic investigations and that the method itself here outlined in principle, will be further developed.



EXPLANATION OF PLATE

Fig. 1. Brassica juncea "wild mustard."
Fig. 2. Linen cloth. Illumination evenly diffused.
Fig. 3. Scales on wing of butterfly.
Fig. 4. Potassium iodide isometric crystals showing accretion and cleavage planes.
Fig. 5. Bottle cork. Sunlight was used. Illumination evenly diffused.
Fig. 6. Shagreen of Squalus acanthias showing placoid scales. Evenly diffused illumination.

ABNORMAL SPECIMENS OF HELODRILUS CALIGINOSUS TRAPEZOIDES (DUGÈS) AND HELODRILUS ROSEUS (SAVIGNY)[1]

By
Bess R. Green
University of Illinois

Introduction

Comparatively few abnormal earthworms have been described, although variations in both external and internal structures are of common occurrence among the various species of Lumbricidae. The importance of the study of abnormal development in earthworms lies in the application of the knowledge thus obtained to the relations that exist in normal development. In normal development growth proceeds in such a way as to bring about the formation of similar structures in the right and left halves of the same somite. Occasionally the normal method of procedure is interfered with and an asymmetrical arrangement of the organs results. The organs develop from paired germ-bands which arise practically independently on the right and left sides of the body thus making asymmetrical relations between the organs of the two sides possible. In abnormal worms the organs may be present in unequal numbers on the two sides, or the members of a pair of organs many develop in different somites on the right and left sides. In many specimens there is also a lack of certain correlations between external and internal structures that occur in normal individuals.

Variations other than those involving asymmetrical relations are of two types. Abnormal specimens in which organs vary but slightly either forward or backward from their normal positions constitute one group or type. The second group is composed of worms in which the organs appear in other than normal positions due to a doubling of somites in the anterior part of the body. There may also be a lack of symmetry between the organs of the right and left sides in either group. In a recent paper Professor F. Smith (1922) described abnormal specimens of *H. subrubicundus* and *H. tenuis* which illustrate the first type of variation, and this paper treats of two examples of the second type found in *H. caliginosus trapezoides* and *H. roseus*.

A detailed discussion of the literature dealing with abnormalities in earthworms will be undertaken later in a more extensive paper. At present there is not sufficient data to make such a discussion of any great value.

[1] Contribution from the Zoological Laboratory of the University of Illinois, No. 219.

The specimens here described are from Professor F. Smith's collection of abnormal earthworms. These worms were found in the banks of a stream at Urbana, Illinois.

Helodrilus caliginosus trapezoides (Dugès)

The description of this specimen is based on the study of sagittal sections of the first thirty-three somites. The worm resembles the normal *H. caliginosus trapezoides* in the general appearance of the somites and clitellum, in the relative positions of the setae, and in the presence of paired, ventral, glandular pads on three of the anterior somites.

External Characters.—This worm which appears to have been injured at the posterior end measures 11.1 cm. in length and there are 130 somites present. There is no evidence of somites that are doubled on one side and not on the other. The setal arrangement in somites 24 to 30 is indicated by the formula $aa:ab:bc:cd = 11:1.5:7.2:1$. This is about the usual arrangement for the setae in the species. The saddle-shaped clitellum extends over somites 32-37 and is very slightly developed on 31 and 38. In a normal worm it commences on 27, five somites farther forward, and extends posteriorly over eight or nine somites. The tubercula pubertatis of the right side is continuous over three somites, 34-36, and the other one involves three and one third somites, 2/3 33-36. In a normal individual they are present on somites 31-33. They have the usual relation to the clitellum since they end posteriorly on the somite immediately in front of the one on which the clitellum ends. The glandular papillae which usually include the ventral setae of somites 9, 10, and 11 are found in this specimen on 10, 11, and 12 on the right side and on 11, 12, and 13 on the left. The setae included by these papillae are modified and are about twice as long as ordinary setae and are much more slender. The first dorsal pore is present on 14/15 which is five somites posteriad of its usual position. A spermiducal pore surrounded by a glandular papilla is present on 18 on the right side. A similar papilla is present on each of somites 20 and 21 on the left side and surrounds what appears to be a spermiducal pore on each of these somites. Normally there is a pair of pores on somite 15. An oviducal pore is present on 17 on the right side, and there is one on 19 on the left. These positions are respectively three and five somites posteriad of the normal positions of the pores. On the right side, spermathecal pores are present on 11/12 and 12/13, two somites posteriad of their usual positions. The spermathecal pores of the left side are on 14/15, 15/16, and 16/17. The first two pores are situated five somites posteriad of their usual positions, 9/10 and 10/11.

Internal Characters.—Most of the reproductive organs of the right side of the worm are present in normal numbers but vary from their usual positions (Figs. 2 and 3). Spermaries are present in 12 and 13; sperm

sacs bearing the usual relations to these organs are in 11, 12, 13, and 14; and spermathecae are included within the septa 11/12 and 12/13. These organs are situated two somites posteriad of their respective positions in a normal worm. The spermiducal pore is on somite 18, which is three somites posteriad of its normal position. Likewise the ovary, in 16, and the oviducal pore, on 17, are present three somites posteriad of their normal positions. The separation of the most posterior spermary and the ovary by two somites indicates a probable doubling of what would be somite 12 in a normal worm. Further evidence of the doubling of this somite is shown by the presence of two vessels uniting the lateral longitudinal vessel with the dorsal vessel, one in 14 and one in 15; while in normal worms there is but one such vessel, in 12. A pouch of the calciferous gland, normally in 10, is present in 12 on the right side of this specimen. It bears the usual relation to the first spermary being included in the same somite with it.

The reproductive organs on the left side are found five somites posteriad of their respective, normal positions. Supernumerary organs in excess of the normal number will be mentioned in connection with the account of certain reproductive organs and of the "hearts." The spermaries and spermiducal funnels, usually in 10 and 11, are present in 15, 16, and an additional one of each in 17. All three of these spermaries are equally developed. The sperm duct could not be traced to the exterior. Sperm sacs are present in the posterior part of 14, 15, and 16, communicating with somites next posterior; and in the anterior part of 16, 17, and 18, communicating with somites next anterior. An ovary and an oviducal funnel are present in 18. The oviduct extends from the oviducal funnel to the oviducal pore on 19. The usual position of the ovary and oviducal funnel is in 13 and that of the oviducal pore on 14. The spermathecae, three in number, are included within septa 14/15, 15/16, and 16/17. Normally there are but two spermathecae present, within septa 9/10 and 10/11. The pouch of the calciferous gland is very slightly perceptible, if present at all, in 14. The position of the pouch is not in conformity with its usual relation to the most anterior spermary. Ordinarily these two structures are present in the same somite.

There are seven "hearts" on the right side and nine on the left. The most posterior "hearts," which are usually in somite 11, are in 17 on the left and in 13 on the right side. As above mentioned two vessels arise from the dorsal vessel in somites 14 and 15 on the right side and join to form a single lateral longitudinal vessel. A lateral longitudinal vessel was not seen on the left side. Normally a pair of these vessels joins the dorsal vessel in the somite next posterior to the one in which the most posterior "hearts" are found.

Helodrilus roseus (Savigny)

The following description is based on transverse sections of the first twenty-three and twenty-four somites and on frontal sections of the ventral half of somites 24 and 25 to 35 and 37. The fact that the spermathecal pores of this worm are located near the mid-dorsal line eliminates from consideration all species of this part of the country except *H. foetidus* and *H. roseus*. Since this worm does not have the bands of color which are characteristic of *H. foetidus*, it is assumed to be an abnormal specimen of *H. roseus*.

External Characters.—On account of the doubling of certain somites on one side and not on the other the somites have been numbered on both sides independently. Posterior to 12 the left half of each somite bears a different number from that of the right half. The relative positions of the various organs are shown in the accompanying diagram. The location of the different organs indicates a probable doubling on both sides of most of the somites in the anterior part of the worm.

Four somites, 13, 34, 39, and 66, are double on the left side; and 100 and 111 are double on the right side. The total number is 162 on the right side and 164 on the left. The maximum number recorded for a normal worm of this species is 150. The setae bear the usual relations to each other. Setae *c* and *d* on 16, 18, 21, 22, and 23 on the left side, on 20 and 21 on the right side, and setae *a* and *b* on 16 on the right side are about twice as long as the ordinary setae. Glandular swellings surround these setae. In normal worms setae of one or more bundles of 9, 10, 12, or 13 may be similarly modified. The clitellum, which is not very pronounced, is saddle-shaped and does not include the ventral setae. It is developed on fifteen somites, while in normal specimens it usually includes but eight. These somites are identical for both sides, although the somite numbers differ on the right (45-59) and left (48-62) sides. Each of the tubercula pubertatis is divided into two distinct areas. The one on the left side includes a smaller part which extends over the posterior two thirds of 54 and the anterior one half of 55, and a larger part which extends from the posterior two thirds of 56 to about the middle of 59. Of the one on the right side, the larger part is more anterior reaching from the posterior two thirds of 52 to the middle of 55; and the smaller part extends from the anterior margin of 57 to the middle of 58. In the normal worm the ridge of each side is continuous and extends over but three somites, 29-31.

Glandular swellings are present surrounding setae *ab* on somites 27 and 28 of both right and left sides of the worm. On external examination there appeared to be a pore in the middle of each swelling, but a study of sections of this region showed but a single pore on each side situated on the anterior of the two glandular swellings. These are the spermiducal pores, present on 27 of each side. In a study of the sections two pairs

of oviducal pores were found on 24 and 25 of both right and left sides. These pores are close to setae *ab* and are very inconspicuous. The spermathecal pores of the right side are present on 16/17-19/20, and those of the left side are on 15/16 and 17/18-19/20.

Internal Characters.—Evidence of the doubling of somites is shown by the presence of twice the normal number of spermaries, spermathecae, and ovaries, and also by the large number of "hearts" that are found in this specimen (Figs. 1 and 2). All four spermathecae of each side are normal in form, and their pores are situated in the usual position near the mid-dorsal line. Spermaries and spermathecae of the same side are present in the same somites, 17-20 on the right side and 16, 18, 19, and 20 on the left. This is the usual relation of these organs. This relation is shown very clearly on the left side; where the first spermary is separated by one somite from the second, and the spermathecae of that side are likewise separated. Another striking relation is shown in the development of lateral pouches from the calciferous gland. Normally a pouch develops on either side of the oesophagus in the somite in which the most anterior pair of spermaries develops. In this specimen the first spermary on the left side is in 16, and a calciferous gland pouch is also present in that somite. The second spermary, the first of a series of three, is in somite 18, and a calciferous gland pouch is also present in that somite. On the right side, the spermaries are present in somites 17-20, and a pouch is found in the somite with the most anterior spermary. A third relation is shown by the relative positions of the sperm sacs and the spermaries. It is usual in this species for two sperm sacs to develop from the septum which bears the second spermary, one from the anterior face and one from the posterior face. This relation exists in this specimen in connection with the septum numbered 18/19 on the left and 17/18 on the right. This septum bears the second spermary on the right side and the second of the series of three on the left side. The spermiducal funnels show normal relations to the spermaries and sperm ducts. Two well developed ovaries are present on both sides, each having the usual relations to an oviducal funnel, ovisac, oviduct, and oviducal pore. The ovaries on each side are situated in the third and fourth somites posteriad of the somite which contains the most posterior spermary of that side.

Eleven "hearts" are present on each side of the alimentary canal in somites 10 to 12 inclusive. The "heart" in somite 12 on the left side is very small. The most posterior "heart" on each side is in the same somite with the most posterior spermary of that side. This is the usual relation of these organs. A lateral longitudinal vessel unites with the dorsal vessel on either side in the usual position, which is in the somite next posteriad of the one in which the most posterior "heart" is found.

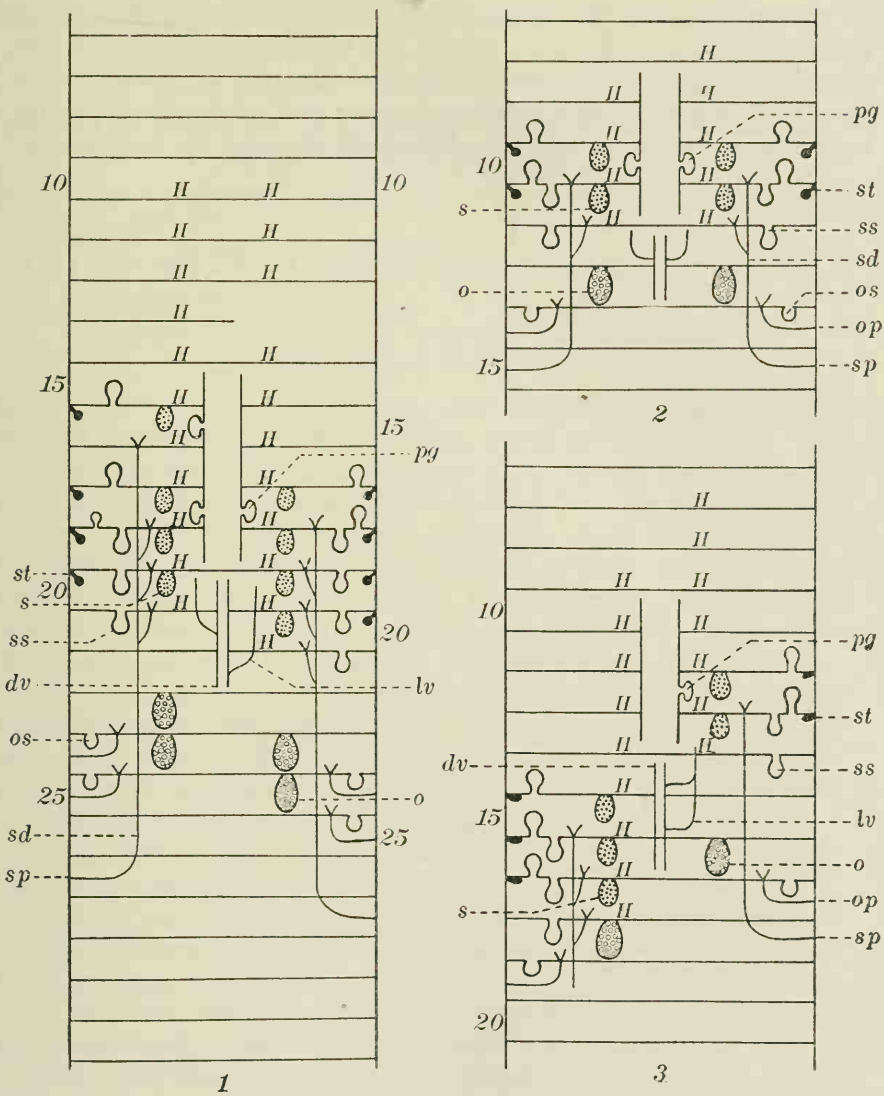


FIG. 1. *Helodrilus roseus*, abnormal specimen: *dv*, dorsal blood vessel; *H*, "heart"; *lv*, lateral longitudinal vessel; *o*, ovary; *os*, ovisac; *pg*, pouch of calciferous gland; *s*, spermary; *sd*, sperm duct; *sp*, spermiducal pore; *ss*, sperm sac; *st*, spermatheca.

FIG. 2. *Helodrilus roseus*, normal specimen: *op*, oviducal pore. Other letters as for figure 1. This diagram also represents the arrangement of the organs in a normal *Helodrilus caliginosus trapezoides*, with one exception. In the latter species the spermathecae are included within the septa and do not extend into the cavities of the somites.

FIG. 3. *Helodrilus caliginosus trapezoides*, abnormal specimen: *op*, oviducal pore Other letters as for figure 1.

A comparison of this specimen with a normal one naturally leads to the assumption that for some reason, not yet obvious, there has been some disturbance in developmental processes which has led to the development of two somites with contained organs from each of most of the units of developing tissue which would normally give rise to a single somite.

DEPARTMENT OF METHODS, REVIEWS, ABSTRACTS, AND BRIEFER ARTICLES

A STUDY OF THE STABILITY OF STAINING SOLUTIONS

By
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Very early in the writer's work in plant histology, he was confronted with the statement that most staining solutions would change their characteristics with age and that many would become entirely useless within a period of a few months. With such conditions true, the duplication of specific staining results would be impossible except through the use of freshly prepared solutions. Even the use of some old and reliable solutions would be out of the question. In many cases the careful comparison of methods of staining is of importance. It is often desirable to exactly duplicate a process of staining. Individual workers find it burdensome or even impossible to prepare stains anew for each new piece of work, or even to prepare new solutions two or more times within a year. A study of the more common stains and their solutions suggested no reason for their breaking down, so a program of experimental work was planned to determine whether or not such a breaking down was necessary, and if so what was its cause. The results of the work have been so gratifying that it seems wise to pass them on to other workers.

Most of the solutions were prepared in 1912. Extreme care was used in cleaning all glass ware and utensils used in the work. Gruebler's dyes were used, only chemicals of a reagent grade, chiefly Merck's Blue Label, were used, and only carefully distilled water was used. The solutions were stored in glass-stoppered bottles. No attempt was made at any time to produce other than normal storage conditions. The bottles were kept in an ordinary cupboard in a laboratory room where a wide temperature range (5°-40°C) prevailed. From time to time the bottles were opened for withdrawal of solutions for individual and class use. No special seal, other than well ground stoppers, was used.

The dyes and chemicals used in the early preparation of solutions were those considered as of a high grade at that time. There has not yet elapsed a sufficient period for a positive statement as to the action of dyes produced by American manufacturers since the war period, but the writer's experience within the available time has given promise of results

fully as satisfactory or even better than the results obtained with Gruebler's products. Many brands of tested reagent chemicals have also proven entirely satisfactory.

It is the writer's opinion that most, if not all, of the trouble in the keeping of common staining solutions is due to carelessness in preparing the solutions, to the use of poor dyes and chemicals, or to the introduction of foreign matter in water or from poorly cleaned utensils and containers.

The following annotated list gives the results obtained by the writer from the storage of various staining solutions through a considerable period. Where no date appears, the solution was prepared in 1912.

Brazilin, (Saturated solution of crystals in absolute alcohol.)—Grown quite dark brown, but far more vigorous than when fresh as used in various formulae as for Haematoxylin.

Carbol-Fuchsin, Ziehl-Nielson.—Filtered repeatedly to remove slight precipitate, but clear and vigorous as ever. (1904.)

Borax-carmine, Grenacher.—As good as new in every way, and more quickly staining.

Borax-picro-carmine, Baumgarten.—Just as new.

Ammonia-carmine, Beal. (Clark, Practical Microscopy, 230.)—Just as new.

Ammonia-carmine, Gerlach. (Clark, supra, 230.)—Just as new.

Chloral-carmine, A. Meyer.—Just as new.

Litho-carmine, Orth.—Just as new.

Para-carmine, P. Mayer.—Always a vigorous and beautiful stain. Increases vigor and intensity with age.

Carmalum, P. Mayer.—Has had to be filtered several times, and is noticeably weakened. (1904.)

Ammonia-cochineal, Pickett. (Science, Oct. 11, 1912.)—Considerable precipitate on sides of bottle, but solution clear and vigorous.

Congo Red. (Equal parts of alcohol and saturated solution of Congo Red in aniline water.) —Just as new.

Cyanin. (1 gm. Cyanin dissolved in 100 cc. alcohol to which 100 cc. aniline water is then added.)—Became almost inactive in 1921.

Fuchsin. (Equal parts of alcohol and saturated solution of Fuchsin in aniline water.)—Just as new. (1913.)

Gentian Violet (Saturated solution in clove oil.)—More vigorous than when new.

Haematoxylin (Saturated solution in absolute alcohol.)—Has become very dark brown in bottle about half full. Used in making up Delafield, Erlich Acid, and Kleinenberg solutions it hastens "ripening" greatly, so such solutions may be used within a few days. Distinctly more valuable than fresh material.

Haematoxylin, Erlich Acid.—Has become a very dark crimson color. Heavy precipitate on walls of bottle, but solution far more vigorous than when new. Tends to precipitate in alcohol more than a fresh solution. (1909.)

Haematoxylin, Grenacher.—Filtered several times and considerably weakened. (1904.)

Haematoxylin, Delafield.—Precipitate on bottle sides. Weakened somewhat since 1920.

Haematoxylin, Kleinenberg.—As new, with slight precipitate on sides of bottle.

Magdala Red, (1% of dye in alcohol.)—Just as new.

Methyl Blue, (Equal parts of alcohol and filtered solution of 2 gm. Methyl Blue in aniline water.)—Slight precipitate. Otherwise as new.

Orange G., (Saturated solution in clove oil.)—Just as new.

Saffranine I., (Equal parts of saturated solution of alcohol-soluble Saffranine in alcohol, and aniline water.)

Saffranine II., (Equal parts of saturated solution of alcohol-soluble Saffranine in alchol, and of water-soluble Saffranine in aniline water.)

Saffranine III., (Same as II. but with distilled water instead of aniline water.)

Saffranine IV., (Equal parts of alcohol and saturated solution of water-soluble Saffranine in aniline water.)

All Saffranine solutions are clear and vigorous as when new.

MODERN MICROSCOPY, A HANDBOOK FOR BEGINNERS AND STUDENTS. By M. I. Cross and Martin J. Cole. Fifth Edition. Revised and rearranged by Herbert F. Angus. With Chapters on special Subjects by various writers. Chicago. Chicago Medical Book Co., 1922. X +315 pp.

This book is written for the beginner in microscopy in England or, judging from certain portions of it, one might perhaps say London. In looking through its pages one might well infer that the manufacturing opticians of London were the only ones in the world. Nowhere in its pages is there a reference to the products of the great optical works of Zeiss, Leitz, Reichert and others in Europe or of Bausch and Lomb or Spencer Lens Co. in America. As a further indication of the public for which the book was written Appendix III, Microscopical Societies and Clubs might be cited. Here four societies and clubs are discussed, of which three are located in London and the fourth in Manchester. The societies in the provinces are dismissed in four lines while the societies in the remainder of the world get no attention whatsoever. If the user of the book will, however, overlook these deficiencies and will provide himself with the catalogues of the dealers and manufacturers of this country he cannot help finding in it much useful information.

The book is divided into three parts which will be reviewed in order. Part I includes eleven short chapters and three appendices. It deals with the microscope and accessories, their construction, and use. The descriptions are characterized by their brevity and clarity of expression, and are well adapted to the needs of the beginner who might be overwhelmed by technicalities if the subject was fully presented. Many more experienced workers could read portions of this part with profit.

Part II—The Microscope and the Scientist—comprises seven chapters, of which three deal with the microscope in medicine and one each with the microscope in histology, geology, engineering, and agriculture. The first four chapters of this part give some directions as to methods but the remaining three are chiefly descriptive. All of these chapters are of value to the beginner in that they show many of the applications of the microscope to certain important fields of science. The treatment of each field is necessarily incomplete. Nevertheless, it is sufficient to give a viewpoint to the user of the book and to demonstrate that there are many unsolved problems requiring the use of the microscope in their solution.

Part III—The Microscope and the Naturalist—with its introduction and six chapters is very useful to the beginner who is largely self-trained,

or to the worker who is trained in a very narrow field. The introduction by Wilfred Mark Webb strives to create in the amateur a desire to investigate the things about him and it hints at the wealth of objects for study in any environment. The chapter on Pond Life by the late C. F. Rousselet has admirable notes on methods of collecting, preliminary examination and keeping, apparatus for microscopical examination, preserving and mounting. There is also a collector's calendar which doubtless is of considerable value in England and is suggestive of what might be done for various regions of America. In subsequent chapters, each by a specialist, a somewhat similar treatment is accorded to the fresh-water mites, the foraminifera, mosses and liverworts, and mycetozoa. There is a final brief chapter by M. J. Cole entitled 'Mounting Common Objects.' This, together with the chapter on 'The Microscope in Histology' and the three chapters on 'The Microscope in Medicine,' gives the beginner, especially if he be inclined toward biology, considerable practical information.

The book is well indexed. Typographical errors are not abundant and are not serious. One notes particularly, however, the word 'Ashe' on page 63 misprinted for 'Abbe.'

The reviewer believes that the authors and publishers are to be commended for having made available to amateur and student microscopists the information contained in this book. He feels that, like the amateur naturalists, the amateur microscopists have been altogether too much neglected by those who have had special training and wide experience in the various fields of research involving the use of the microscope. At the same time he feels that this book might have been made very much more useful for its American public had portions of it been rewritten with this public in mind.

GEORGE R. LA RUE

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A STUDY OF THE MOVEMENTS OF ENTOPROCTAN BRYOZOANS

By
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When the bits of rock or shell covered with living *Barentsia* or *Myosoma* are lifted from the water, a very marked movement of all or nearly all the zooids is evident. In the case of the first of these, the characteristic incomplete or partial rotations of each individual are seen to be caused by contractions of the swollen muscular base of each stem. With *Myosoma* the movements differ slightly because the whole stalk is muscular and flexible. The observations in this paper are chiefly upon *Barentsia gracilis* Hincks which is common at Laguna Beach. The species determination was made by Dr. Robertson, whose extensive papers on Pacific Coast Bryozoans are the chief source of information for the identification and distribution of western forms.

The questions which suggested themselves at once when these forms were examined were; what are their movements, how are they stimulated to move, and what relations do the nervous system and sense organs bear to these activities.

The most evident movements of *Barentsia* are those which carry the body with its tentacles through a wide arc by the contractions of the muscular base of the long stalk. These movements tend to describe a complete circle but if obstructions are met with, the rotation ceases and may not be continued unless the zooid is further stimulated. Movements of the sort just mentioned tend to persist in one direction when once started, although at other times the contractions may cause a rotation in the other direction. The next most marked change is seen in the contraction of the tentacles. When a mass of the colony is lifted from the water or otherwise very strongly stimulated, the tentacles contract and remain in this condition for some time even though the other activities are as marked as ever. The body of each zooid is attached to the stalk by a small area of flexible tissue and under certain conditions this permits of a slight rotation of the body upon the stalk.

The work of the past upon this group of Bryozoa gives little hint of their behavior or the nature of their activities but many writers have some suggestion of the structure and arrangement of the nervous system, and for that reason a brief review of the literature follows.

Van Beneden, 1845, considers *Pedicellina* but gives little indication of the nervous system. Kowalevsky, 1867, considers the development. Uljanin, 1869, gives the position of the central ganglion in this genus. Nitsche, 1875, shows the general position and chief branches of the central nervous system in *Pedicellina*. Salensky, 1877, indicates the general location of the ganglion in *Loxosoma*, and Harmer, 1885, gives the most complete early account of the nervous system. In *Loxosoma* he describes a dumb-bell shaped ganglion with nerve cells on the outer surface of the central fibrous part. There are many bipolar sense cells, especially in the tentacles which send their fibers into the brain. Foettinger, 1887, represents the brain of *Pedicellina* by two more or less separate lobes. Several pairs of nerves pass from the ganglion. Seeliger, 1890, gives the development and position of the nervous system. Davenport, 1893, shows the position of the ganglion in *Unatella*. Nickerson, 1901, in *L. davenporti*, describes the brain as just in front of the intestine and above the stomach. It is elongated transversely. From each end two bundles are given off, one on each side to pass to the lophophore. Sensory bristles were seen on the tentacles. Stiasny, 1905, shows the ganglion of *Pedicellina* but with no detail. Retzius, 1905, shows sensory nerves on the surface of *Pedicellina*. These sensory cells bear bristles and are connected with nerve strands which form a wide network of fibers. Sensory cells were marked in the tentacles. Assheton, 1912, found the nervous system in two species of *Loxosoma*. The brain and chief branches are figured, and sense cells are mentioned on the hypostome, lophophore and tentacles.

In *Barentsia* and *Myosoma* the central ganglion is small. Its connection with other parts was not traced, but after trials during several summers sense cells were demonstrated in the tentacles, and especially at the tip of the stem where it joins the body of the zooid. Sense cells and a plexus of nerve fibers or a nerve net were demonstrated, especially forming a network in the unswollen part of the stem. Bipolar nerve cells of the stem were demonstrated sending their fibers into the sense pores or the little pits in the cuticle of the stem. These pits occurred only on the unswollen parts of the stem. Methylene blue was used to show the sense cells and the nerve net.

In *Myosoma*, instead of sense pits the stems were covered with hollow sensory hairs much like those so common in arthropods, but in this form no demonstration of the sensory cells was made. Although there are no sense cells in the swollen base there is a very marked nerve net under the cuticle in *Barentsia*.

So far as I have been able to determine, there is no account of the activities of *Barentsia* and no experiments to determine their control. In *Loxosoma* the movements are quite different, partly because of their not being colonial forms and partly because of their different form and structure, so no time will be taken to discuss them further. *Barentsia* has a creeping stolon which is often hidden by other growths, but sometimes it may readily be seen upon the surface of a rock or a bit of sponge and it is evident that there is great variation in the way in which the individuals of the loosely formed colony are linked together. Each individual of a chain or network has its own part of the stolon cut off from the rest by well-marked partitions across the stem.

In summarizing the general effects of various stimuli upon the movements of *Barentsia*, the two minor movements may be dismissed with a brief statement. The tentacles do not withdraw themselves unless the stimulus is quite severe or sudden. They may be withdrawn when the animals are removed from the water, they contract when touched rather directly and they do not change much in relation to light or chemicals either when expanded or contracted.

The rotations of the body on the stem at the point where the stem joins the body are not very marked at any time, but they seem to be more evident with freshly obtained individuals that are strongly stimulated especially by touch or jar, although they were occasionally seen to move in this way when no exciting cause was evident.

The response which is most marked and which is chiefly considered in the following account is the rotation due to contractions of the swollen base of the stem or stalk. The maximum reaction is a movement of 360°. Freshly obtained specimens tend to perform this greatest movement and although after a time there may not be quite so great a swing, yet the members of the colony remain active for a long time. This activity is not primarily due to stimuli coming to the body as is shown by entirely removing it from the stem, for then the rotations are as marked as before. Tactile stimuli were used in large part because these could be more definitely localized than any others. It was found that the most sensitive areas were first the tip of the stem where it joins the body, and next the stem or stalk. Even the swollen base of the stem is more sensitive than the body even though no sensory endings were demonstrated in it. As a jar is one of the most powerful of the stimuli, it may be that this explains the reason why the fleshy base may be stimulated to cause the movement and why the body because of its flexibility does not transmit the jar so well. Besides tactile stimuli which have always an element of jar, currents in the liquid are also effective. There is but slight response to light either continuous or intermittent. They seem to move a little more in the brighter light. There is also but slight response to heat and if the tempera-

ture is gradually increased they may die without movement. Rather weak acids cause a sharp response but stronger ones or strong poisons may kill them quickly with little evident reaction.

It would seem probable that under natural conditions where the colonies are hidden in dark crannies of rock surfaces that stimuli in the nature of contact, pressure, or currents would be the ones to bring about the marked movements which may be something in the nature of avoiding or freeing reactions. The violent movements induced by removing the zooids from the water may be due to the unwonted weight brought upon the stem by the rather heavy bodies.

As a jar seems to be the most effective in bringing about a response when the zooids are touched, it may be that the tactile stimuli employed have an element of jar and either the jar or a combination of effects from jar and touch both bring about the responses. The creeping stolon is very sensitive to the least touch as is shown by the activity of one or several stems, but here even more than on the stems or their swollen bases is it difficult to discriminate between jar and touch. However, living stolons seem to carry the responses better than dead portions. A nerve net was demonstrated in the stolons but it was impossible to be sure that the nerve actually crossed from one member of the colony to another across the partition in the creeping stem. If the stolon is stimulated near where several parts join there may be a very active response in the way of rotation in a number of nearby zooids. Once when a stem was cut through there seemed to be a shock to a number of zooids for they did not respond so well for a time when stimulated through this cut portion.

In most cases tactile stimuli were tried by means of a needle point. During the observations zooids were kept in fresh sea water under as normal conditions as possible. In most cases the stems or muscular bases of the stems were touched, but in some expanded individuals the tentacles were touched. The usual response was a slight contraction of the tentacles stimulated near or on that side and a slight movement of the body on the stem. Repeated stimuli cause the tentacles to draw in more. If the center of the tentacles is touched there are more violent movements of the tentacles and of the body but the stem may not rotate unless the touch is quite heavy. Small regenerating bodies are more sensitive and the stems may be induced to rotate when these are stimulated. When the body is removed, the cut end of the stem is especially sensitive and when touched the stem rotates violently.

When a zooid is stimulated to begin a movement further stimuli even from several directions do not as a rule change its direction or movement for some little time.

When an individual is touched lightly it may not move; if light stimuli are continued it may begin to move after three or more light stimuli.

If light stimuli are continued it may move away rather more violently. When a specimen has been caused to move a number of times it may become fatigued and not move again for a period which differs in different individuals. Small specimens often respond more quickly than large ones.

When a zooid is strongly stimulated the effects may be carried to more distant zooids, and a whole chain or a large group may be caused to move by the movements of one. A stimulus to one zooid may not produce movement in it but others more distant may be affected. In some cases there seems to be a blocking of an impulse when intermediate zooids do not respond as for instance when the intermediate one is dead or fatigued. A stimulus to a moving zooid apparently may not increase its activities but more distant individuals may start to move although they have just ceased moving. In this apparent blocking of impulses there is quite a little chance for error in interpretation because a slight jar or lack of it may be hard to determine in individual cases. In respect to fatigue there seems to be little question that the results are as indicated. In regard to the apparent summation of stimuli before a response, it might be urged that the stimuli were not all even and the last one given was heavier than the rest but careful checking of this seems to justify the result obtained.

Various anaesthetics were used on the living specimens. Chloral hydrate or Chlorotone were used until there was no longer any response. The body and tentacles were first to lose their sensitiveness and the last to regain it.

General Conclusions

1. Barentsia has four chief motor activities:
   (a) Movement of the zooid as a whole through rotation of the stem caused by contractions of its fleshy base.
   (b) Movement of the body on the stem.
   (c) Movement of the tentacles, contraction and expansion.
   (d) Movement of external and internal cilia.

2. As suggested by both experimental and anatomical methods there are three chief centers of motor control: (a) The ganglion of the body for the control of the tentacles. (b) The plexus or nerve-net where the body joins the stem. This is for control of the movements of the body on the stem. Apparently also, here is a sensory area for receiving and carrying down the stem sensations or movement from the body. Possibly this is the chief connection the body has with the stem, this rather indirect one. In any case the connection is not marked judging from the experiments in stimulating the body. (c) The sensory pits, bipolar cells connected with them and the nerve-net of the stem. Sensations are received here and conducted down the stem. There is little or no evidence of their going in the other direction or affecting the body or tentacles in any way.

(d) The very marked nerve plexus in the swollen base of the stem which receives fibers from the stem above or from the plexus of the creeping stolon and responds to impulses coming over these fibers. The swollen base may receive impulses in the way of jar or pressure directly, but because of the thickness of the cuticle and the lack of special tactile organs it seems not to be so sensitive to light touch as the stem or stolon. It seems to be the center for the control of the larger movements of the stem. (e) The creeping stolon marked off by partitions between zooids. This has sense pits and a nerve plexus similar to the stem above the swollen base.

3. There are then three centers of control: (a) The ganglion in the body for the tentacles. (b) The first part of the stem especially for movements of the body on the stem. (c) The swollen base which is not only a contractile organ but also contains the nerve plexus which controls the larger movements of the stem.

4. It seems possible to fatigue the zooids by continuous movements.

5. Fatigued zooids may block the pathway of a stimulus.

6. Stimuli pass from zooid to zooid along the stolon.

7. Stimuli to the tentacles may cause them to contract without affecting other parts. Rotations of the zooid may not affect the condition of the tentacles if the body does not touch anything.

8. Weak stimuli which do not individually produce a response may be summed and cause a reaction.

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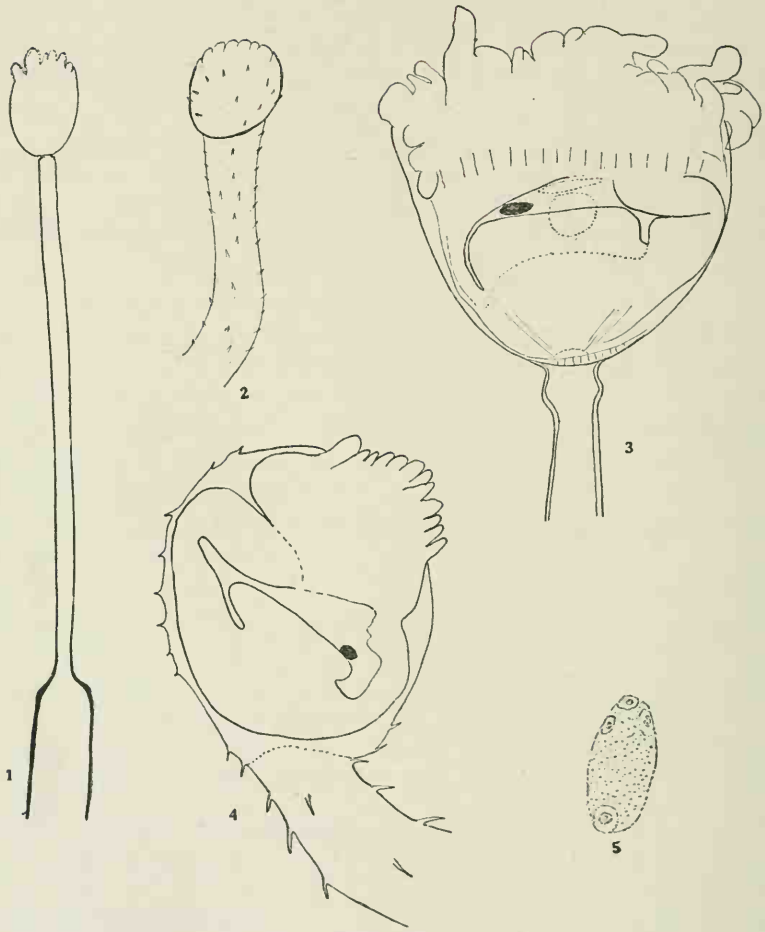

PLATE VI

Fig. 1. Sketch of a single preserved specimen of Barentsia. x17.
Fig. 2. Sketch of a single preserved specimen of Myosoma. x17.
Fig. 3. Zooid end of Barentsia. Nerve ganglion in dark. x35.
Fig. 4. Zooid end of Myosoma. Ganglion in dark. x35.
Fig. 5. Section of the central ganglion of Barentsia. x150.

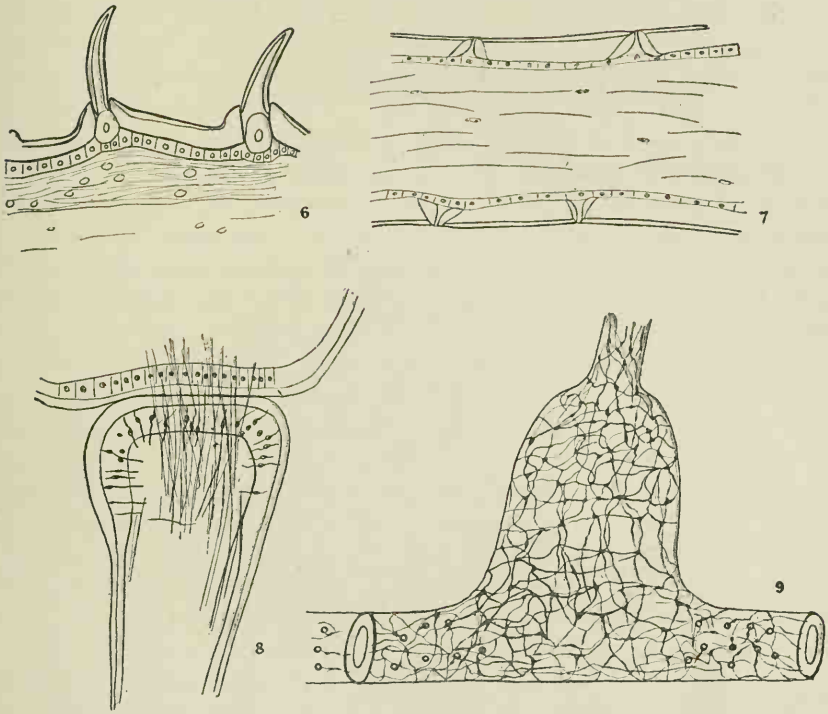

PLATE VII

Fig. 6. Section of the stem and sense hairs of Myosoma. x150.

Fig. 7. Section of the stem of Barentsia. x150.

Fig. 8. Optical section of base of zooid and stem of Barentsia, showing sense cells stained with methylene blue. x35.

Fig. 9. Base of stem of Barentsia stained with methylene blue to show sense pits and nerve network. x30.

OBSERVATIONS ON THE LIFE CYCLE OF DAVAINEA PROGLOTTINA IN THE UNITED STATES

By

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In the course of parasitological examinations of slugs, *Agriolimax agrestis*, made on January 23rd, one specimen out of about 25 collected from a chicken yard contained very large numbers, 150 or more, of tailless cysticercoids which morphologically resembled the scoleces of *Davainea proglottina*. The heart-shaped cysticercoids measure from 230 x 160μ to 160 x 140μ and possess a gelatinous wrinkled outer coat, varying in average thickness from about 14 to 30μ, but always thicker at the posterior end. The variation in size of the cysticercoids is due principally to the thickness of this outer coat. The wrinkled or corrugated surface reminds one somewhat of the gelatinous coat of an Ascaris egg. A rather heavy fibrous wall delimits the heart-shaped inner body of the cysticercoid which contains large numbers of very refractile calcareous corpuscles averaging about 10μ in diameter. The withdrawn scolex measures about 80μ in diameter, but as a rule its outline is very indistinct, being obscured by the calcareous granules. The single rostellar circlet of about 75 to 90 hooklets is very distinct; the hooklets measure about 8μ in length. The suckers themselves are readily distinguishable by the single circlet of hammer-shaped hooklets.

Some of the cysticercoids were found free in the body cavity of the slug, but many of them were imbedded in the tissues, particularly in the walls of the alimentary canal.

Portions of the slug containing numerous cysticercoids were fed to two hens, each about two and a half years old, which by fecal examination were found to be free from any species of Davainea, although the eggs of another cestode, which was subsequently found to be *Amoebotaenia sphenoides*, were found. These birds came from a flock of six hens which had been kept away from other poultry for over a year, and two other members of the flock which were examined at other times for parasites were also found to be free from any species of Davainea.

On February 12th, twenty days later, the droppings of the hens were examined and found to contain active proglottids of *Davainea proglottina* in considerable numbers. It is probable that the worms had matured some time before, inasmuch as Grassi and Rovelli (1889, 1892) found that the adult stage was reached in chickens eight days after ingestion of infected slugs. Grassi and Rovelli's work was not at hand at the time, and

it was not realized that development would take place so rapidly. One of the hens was killed and found to contain about 50 specimens of *Davainea proglottina* and numerous individual proglottids. The droppings of the other hen were examined at intervals of three or four days to determine how long the active proglottids would continue to be voided. On Feb. 21. only a single proglottid could be found in a night's droppings, two were found on Feb. 24, and none at any time after that date, which would indicate that in birds of the age of these hens the infection is not of long duration.

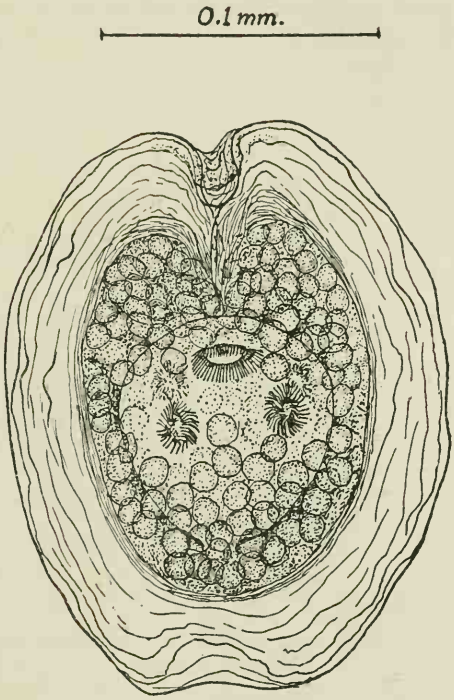

FIG. 1. Cysticercoid of *Davainea proglottina* from naturally-infected *Agriolimax agrestis*

A number of living proglottids were fed, on lettuce leaves, to two laboratory bred slugs, *Agriolimax agrestis*, on Feb. 12, and a number of others were fed to four other laboratory bred slugs on Feb. 13. The latter slugs were later found to be of another species, *Limax flavus*, identified by Dr. H. A. Pilsbry, and said by him to be the first record of its occurrence in the southwest.

One specimen of *Agriolimax agrestis* was examined on Feb. 22, ten days after infection, and found to contain about twenty young larvae, in a very early stage of development. These larvae measured from 127 x 98μ

to 145 x 118μ but were practically undifferentiated, showing no signs of invagination. The embryonic hooks, about 16μ in length, were still present, situated as shown in Fig. 2. No indication was found of a vestigial tail, such as is described by Grassi and Rovelli. The other specimen of *Agriolimax agrestis* was examined on March 6, 22 days after infection, and found to contain cysticercoids similar to those found in the naturally infected slug.

Two of the four specimens of *Limax flavus* which were fed proglottids of *Davainea proglottina* on Feb. 13, were examined on Feb. 27, 14 days after infection. One specimen was entirely negative, while the other contained a single cysticercoid measuring 213 x 213μ. This specimen is heart-shaped, has invaginated, and shows a retracted scolex, but there is no indication of rostellar hooks, suckers, or acetabular hooks, nor of a vestigial tail. The

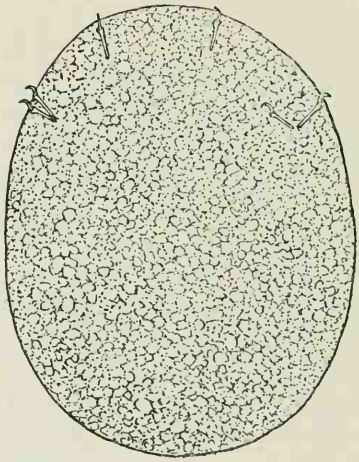

FIG. 2. Young larva of *Davainea proglottina* from *Agriolimax agrestis*, 10 days after laboratory infection

calcareous granules are much smaller than in the fully developed cysticercoid, and the entire specimen has a coarsely granular appearance. A third specimen examined on Mar. 3, 18 days after infection, was also found to be entirely negative. The fourth specimen escaped.

It would appear from the above experiments that *Agriolimax agrestis*, which is one of the species which Grassi and Rovelli were successful in infecting, serves as an intermediate host in the United States as well as in Europe. *Limax flavus* would appear to be highly resistant to infection, since only a single cysticercoid developed out of hundreds of eggs fed to three individuals, and it is not probable that this species would serve as an intermediate host in nature.

The occurrence of *Davainea proglottina* in the United States has previously been recorded only by Ransom (1909) from Pennsylvania and Maryland. Guberlet (1916) states that this species has not been recorded in the United States, evidently having overlooked Ransom's records, which are cited only in a footnote. His erroneous statement has been copied in text books and has led to the general assumption that this species has never been introduced from Europe. Its occurrence in such widely separated regions as Pennsylvania, Maryland and Texas indicates a rather wide distribution in this country.

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THE EGG LAYING HABITS OF HAMINEA VIRESCENS (SBY)*

By
A. Richards

The green bubble shell, *Haminea virescens* Sowerby, belongs to the opisthobranch gasteropods of the family Bullidae. The genus occurs in suitable locations on both Atlantic and Pacific Coasts. According to Gould it is "common in the muddy lagoons and salt ponds along the shores of Vineyard Sound" and elsewhere in the Woods Hole vicinity. This species may be found along the southern California coasts on tidal flats burrowing into the sand at low tide.

Embryological phases of three species of this genus have been studied. Smallwood in 1901 reported observations on the behavior of the centrosome during maturation of the eggs and in 1904 described in detail the maturation, fertilization, and early cleavage of *H. solitaria*. In another paper of 1904 he discussed the natural history of the species, including the egg lay habits. The species found in Puget Sound is *H. vesicula*. Its early development was reported by Leonard in 1918, and Bovard and Osterud include a note on the form of the egg masses in their paper of the same year. Finally, the writer described briefly in 1922 the habits of *H. virescens* obtained at La Jolla, California, and gave an account of experiments performed on them.

The accounts of the egg masses of these three species are by no means in agreement. For this reason it is desirable to bring together the descriptions of all three, pointing out in detail the condition observed in *virescens*.

During the summer of 1921 while working at the laboratory of the Scripps Institution for Biological Research at La Jolla, I used the eggs of *Haminea virescens* for experimental purposes. For reasons that will appear in connection with the description of the egg masses these eggs are especially suitable for experiments in which it is desirable to maintain to the greatest possible degree the normal environment of the egg. The animals were brought into the laboratory and kept in a dish of running sea water in which were stones and a quantity of sand from the tidal flat where the specimens were originally obtained. No difficulty was experienced in getting them to produce eggs for a number of days. At length, however, they became exhausted and a new supply had to be obtained. Whether the eggs failed because of a natural course of events or because of the laboratory conditions could not be determined.

Egg laying occurs usually about the time of the first light in the morning. It is not improbable that the light acts as a stimulus of some nature.

* Studies from the Zoological Laboratory of the University of Oklahoma, Second Series No. 36.

I was unable to observe the actual laying process, but from the known facts for the periods elapsing during the cleavage stages and from the comparison with similar phenomenon in other gasteropods, it is probable that egg deposition took place in the early morning between three and four o'clock. Light in this latitude comes during the summer months very early, for the sun rises between 4:30 and 5 o'clock. There are of course exceptions to the rule that egg deposition takes place this early. I have found one cell stages at 6:20 and 6:45 A. M., and in one case when I came into the laboratory late in the afternoon (at 5:30 p.m.) I found an egg mass in the four cell stage indicating that it had been deposited perhaps four hours earlier, or not long after mid day. Perhaps the laboratory conditions may have been responsible for this irregularity. On the other hand, I found 12 cell stages as early as 6:30 A.M. on several occasions and once as early as 5:40 A.M. Nevertheless, usually it appeared that the first sign of light must have been the time of laying. Leonard finds likewise that *vesicula* lays during the early morning hours. She says, "The writer has gathered large numbers of the animals and of their characteristic egg-masses from the under side of the *Ulva* at all times of day. Eggs in the 1-celled stage and apparently freshly laid were obtained most plentifully between the hours of 5 A.M. and 9 A.M., and Haminea is frequently found in the act of laying between these hours. However, one of the animals was found laying as late as 11:30 A.M., and continued laying in the laboratory until 2:35 P.M."

For some animals it has been suggested that a relation of some unknown character may exist between the times of egg deposition and the changes of tides. My observations for *Haminea* are hardly in keeping with this hypothesis. They were extended over a period of two months and showed that the time of laying varies only as indicated above, while the tide of course varied each month through the diurnal cycle.

The duration of the early cleavages is illustrated by one typical case which was followed through in detail. The figures for this case are about the average for all those studied. The observations on these eggs at 21.5°C (which temperature is the upper limit of the temperature range of the sea water in the laboratory during the summer, and the exact reading of the thermometer on the 3rd of August when this observation was made) are as follows:

1 cell stage first observed at 6:45 a.m.	
Maturation completed at 7:45 First cleavage (2 cells) completed at 9:10	1 hr. 25 min.
Second cleavage constriction begun at 9:45 Second cleavage (4 cells) completed at 10.01	51 min.
Third cleavage constriction begun at 10:40 Third cleavage (8 cells) constriction begun at 10:40	59 min.
Fourth cleavage (12 cells) completed at 11:45	50 min.
Fifth cleavage (16 cells) completed at 12:30	40 min.

On the average it appears from this and other data that the early cleavages range in duration from eighty minutes down to forty minutes. The longer cleavages are of course the earlier ones. The veliger stage is reached at the end of about 48 hours. Hatching begins on the fifth day usually and is completed in two or three days more. The percentage of embryos that hatch is very large unless an external factor interferes. Often I have seen 98-100% hatch, while seldom do less than 85% reach this stage.

From Smallwood's records (1904, a) it appears that the duration of the cleavage divisions of *solitara* is less than in the case of *virescens*. According to him the first polar body is given off ten to fifteen minutes after laying and the second thirty minutes later. "The egg segments into two cells a half hour after the second polar body has appeared" . . . "Within thirty or forty minutes after the formation of the two cell stage, the four cell stage is formed." . . . "After not more than thirty minutes, the third cleavage separates the egg into two conspicuous parts, the protoplasmic micromeres and the deutoplasmic macromeres." . . . "The time that intervenes between the formation of the second and third quartettes of micromeres is the same as that for the second and third cleavage."

The structure of the egg case is the chief point in which my observations differ from others which have come to my attention. The egg cases of the genus *Haminea* are usually described as jelly like masses apparently without special form. In the "Catalog of Marine Fauna (of Waters of Woods Hole and Vicinity)," Sumner, Osburn and Cole record a note by Conklin in reference to *Haminea solitaria* to the effect that the eggs are "laid in large jelly like balls, which are fastened by stalks to the sand," and are deposited "August 20th or earlier." Others speak of formless masses of jelly containing the eggs.

Smallwood's account (1904, a) of the process in *H. solitaria* follows. After describing the process of copulation which is of about fifteen minutes duration and occurs some eight to twelve hours previous to laying, he says, "This species lays a single gelatinous mass which is spherical, about three-quarters of an inch in diameter. Its contents are chiefly composed of albumen, which is secreted by the albumen gland. As soon as the albumen comes in contact with the water it swells by the rapid absorption of water, and thus affords a gelatinous protection for the egg. When the eggs first leave the genital groove they are in strings; in a few hours the strings lose their continuity and the eggs are scattered throughout the egg mass. It would be very difficult to count the eggs in a single mass. The size of the capsule varies considerably; as a rule those found on the eel-grass are about a third less in diameter than those laid on the bottom. The egg masses laid in the laboratory were often irregular in shape and much smaller

than those collected from the pond. The specimens in confinement that laid small and irregular masses, often laid a second time without a second copulation. It takes from 40 to 50 minutes for an animal to lay a complete normal egg mass."

The process of egg laying in *H. vesicula* was observed in the laboratory by Miss Leonard, whose description is as follows:

"In the process of laying the eggs are extruded in a flat band about 1 cm. in width from beneath the right side of the mantle, and pushed back so as to extend posteriorly. During this process *Haminea* is moderately extended. At intervals definite contraction of the mantle on the right side occurs, at which time two or more strands of ribbon are pushed out. Due apparently to a slow spiral motion of the female while in the act of laying, the egg-masses are spirally coiled when of sufficient length."

"Each egg is enclosed in a comparatively large capsule, containing a transparent fluid which does not become opaque upon fixation. These capsules are embedded in a clear, transparent, colorless or pale yellow jelly, which is in the form of a flat band about 1 cm. in width and varying from a fraction of a centimeter to 6 cm. or more in length. The bands are attached by their lower border to the substratum. The eggs vary in diameter from 50 to 90 microns and are embedded in the gelatinous band in a continuous spiral line. The eggs when collected in their masses will live and develop normally for several days under laboratory conditions."

"Fertilization is internal, hence the eggs when collected had already been fertilized. None were found at a stage previous to the formation of the first polar body, and none were dissected out of the oviduct to ascertain whether the first polar body is formed before extrusion. No unfertilized eggs were found, and it is a question whether any are laid. In surface view the egg is bright yellow, with opaque yolk granules scattered thruout. After the egg is laid the second polar body is thrown off by the formation of a typical polar spindle."

In their list of animals yielding embryological material, Bovard and Osterud have the following note in reference to *H. vesicula*. "The egg masses are 12-38 mm. in height, and are whitish balloon-shaped bodies, with numerous eggs enclosed in a gelatinous substance. These may be anchored in mud or on eel grass. The masses are best collected between five and nine o'clock in the morning to obtain the earliest cleavage stages." I am unable to explain the discrepancy in the two accounts of the egg mass in this species. It hardly seems however that Miss Leonard's account can be questioned since it is based upon her own direct observations of the animal while laying.*

* Since the above was written I have myself seen egg cases of *H. vesicula* and can substantiate Miss Leonard's statements. The egg case of *vesicula* is in all essentials like that of *virescens*.

While experimenting on the rate of division in the eggs of *H. virescens*, the writer kept the animals in the laboratory for several weeks and obtained many egg cases, so that there could be no doubt as to the accuracy of the identification of the egg masses or of their similarity to those brought from the collecting grounds to the laboratory.

At first glance the egg cases of *Haminea virescens* appear like thickened sections of "gros grain ribbon" ranging in length from 15 to 35 mm., and about 5 or 6 mm. broad, and of a creamy white color. Even when the cases are taken in open water and have become covered with foreign particles they have never given me the impression of a formless mass. Usually one edge of the ribbon is attached to a small stone while the other floats freely in the water. The attachment is made in such a manner that the ends of the ribbon almost form a completed circle. In this position the eggs develop until hatching is completed when the jelly disintegrates and the entire structure falls to pieces.

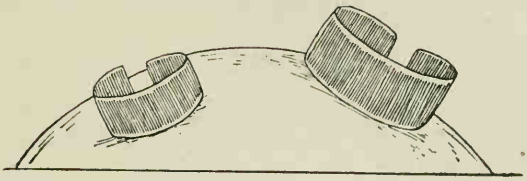
The structure of the egg case is of a complicated pattern, as shown in the accompanying figures. The eggs appear to be extruded in a string of tough gelatinous material which becomes surrounded by the matrix jelly forming the body of the ribbon. Inside of this gelatinous string the eggs are laid alternately so that the appearance is that of a double row of eggs. The string itself is accurately placed in the form of a flattened spiral the loops of which are not formed of back and forth folds as they first appear, but are so arranged that the opposing loops are compressed against each other. This produces the effect of a cross striated thick ribbon.

In one ribbon, that may serve as a typical case, the number of loops producing the striations was counted and found to be 242. In a single length the number of eggs ranged from 80 to 104, averaging 90. There were then in this egg case 21,780 eggs. Probably the number of eggs deposited at one time under favorable conditions ranges from 15,000 to 25,000; at least the data for other normal cases indicate such numbers. When conditions are not so favorable a much smaller number of eggs, even as low as 5,000 is deposited at one time.

One of the most striking facts in regard to these egg cases is that the eggs are always in the same stage of development. Uniformly I found that the entire lot of eggs in a single egg case were in the same stage of mitotic division. It is a remarkable condition that 20,000 eggs should be laid in as complicated a manner as this so nearly at one time that all would divide simultaneously. In these cases however, it was not possible to distinguish by any difference in development which end of the mass was produced first. In other cases where enormous numbers of eggs are shed in the water before fertilization takes place, it is rare to find 100% of the eggs developing, but this is the rule in the cleavage stages of *Haminea virescens*.

Thus it appears that in this genus two entirely distinct modes of

egg protection are to be found. In *solitaria* the egg case is a spherical gelatinous mass of three-quarters of an inch in diameter. In *virescens* the mass is in the form of a narrow ribbon which may be an inch and a half in length with the eggs placed in very regular order. *Vesicula* agrees with *virescens* in the general structure of the egg mass. It is not improbable that the statements of some that the egg cases in this genus are irregular formless masses of jelly are based upon incomplete data.



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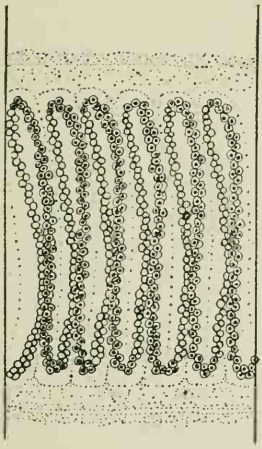

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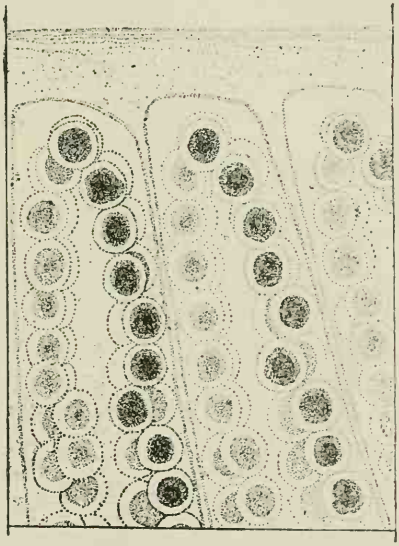

3

EXPLANATION OF FIGURES

Fig. 1. Egg masses of Haminea virescens, in natural position at about natural size.

Fig. 2. Small section of the case showing the arrangement of the spiral loops.

Fig. 3. The upper ends of two loops, showing the eggs in which the spirals are arranged in double rows. Each egg is surrounded by a gelatinous covering within the egg case.

The ribbon like structure of the egg case of *H. virescens* very greatly facilitates the use of these eggs for experimental purposes. The egg case may be cut into sections one of which will serve as a control while others may be placed in various solutions and the results noted. The eggs them-

selves are not disturbed in their gelatinous coverings and the effects of the experiment may be noted without complicating factors. For such purposes the eggs of *Haminea* are ideal.

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DEPARTMENT OF METHODS, REVIEWS, ABSTRACTS AND BRIEFER ARTICLES

NOTE ON THE OCCURRENCE OF THE MARINE CLADOCERAN EVADNE TERGESTINA IN SOUTHERN CALIFORNIA

By
Helen E. Murphy

Dr. Chancey Juday reported the presence of the marine *Cladoceran Evadne tergestina* Claus in Southern California in 1907. He states: "This Cladoceran was found in only eleven catches, eight of which were surface." (Univ. of Calif. Publ. in Zool. vol. 3, no. 10, pp. 157-158.)

An enumeration of three series of catches of zooplankton collected from the surface at 8 a.m. at the Scripps Institution, La Jolla, California, enables me to amplify this brief record. A series extending from July 16, 1922 to May 1, 1923, contained specimens of this species from August to late November, and again in April. The maximum production was in April when there were many free swimming young, and females carrying young. A second, though smaller maximum occurred in October and November. Females carrying young predominated at that time. Two smaller series, September 1916 to January 1917, and September 1919 to January 1920, show adults present from September to January, with immature forms in November.

METHODS OF CULTURING TUBIFICIDAE IN THE LABORATORY

By
Eugene F. Powell

Tubificid worms are important in the laboratory because they are forms whose life history can easily be studied and because they are excellent food for small aquatic animals such as fish, planarians and leeches. *Przibram (1922) states that these worms may be kept for some time in tubs or troughs of muck in which there is much plant detritus and over which water is allowed to flow gently, the temperature being kept low and constant.

* Hans Przibram-Wien: Das lebende Tiermaterial für biologische Untersuchungen (Auswahl, Beschaffung, Haltung unter verschiedenen Bedingungen, Markierung). Aberhalden: Handbuch der biologischen Arbeitsmethoden. Abt. IX, Teil 1, Heft 2. 1922.

The writer started a culture October 9, 1922 for the purpose of study and to provide food for some aquatic animals already mentioned. The worms were obtained by two methods: (1) by washing out some of the muck by the use of sieves and (2) by taking the material as found in a river bed. The latter method resulted in the taking of many more worms. Large glass dishes or pans were used in which the muck containing the worms was placed to the thickness of about two and one half inches. For a time the worms thrived but within a few weeks an appreciable decrease in numbers was noted. Thinking that lack of food was the cause of the decrease some boiled potato was added to the culture and within a few days such good results were seen that a small amount of potato was added about once in every two weeks thereafter. At present (April 30, 1923) the culture is still thriving and the worms are larger and greater in numbers than when taken. It would seem that by the addition of boiled potato or other similar food at frequent intervals a culture could be kept going indefinitely.

A NEW METHOD FOR WHOLE MOUNTS

By
William K. Bowen
Michigan Biological Supply Co.

The destruction of microscopic preparations, due to careless focussing of the microscope, is an occurrence with which biology instructors are only too familiar. In the case of sections, it is often possible to dissolve off a broken cover, and replace with another, but with whole mounts, the specimen is usually ruined. The writer has recently worked out a method, applicable to a large number of whole mounts, which practically eliminates any possibility of injury to the specimen, however hard the objective may be forced down upon the mount.

This method consists essentially in embedding the specimen in celloidin, and in clearing and mounting the celloidin with the specimen within it. The process is briefly as follows: Stain and dehydrate the specimens just as if they were to be mounted in the customary manner. Place for one to several hours in ether alcohol, the time required depending on the size and density of the specimens. Replace the ether alcohol with thin celloidin. Even large and relatively impermeable objects will be sufficiently saturated in 24 hours. Transfer the celloidin and the specimens to a perfectly flat dish or suitable embedding tray. Orient the objects so that when the mass is cut up, each specimen will be surrounded with a mass of celloidin having an area of about half that of the cover glass to be employed. Allow the celloidin to thicken by exposure to the air. When the mass is hardened it should be as near as possible the same thickness as

the specimen. The amount of celloidin to use can be learned only by experience. As soon as the mass has become sufficiently firm, complete the hardening in chloroform vapor. Remove from the mould, and clear. Finally cut the mass up into blocks, each containing a specimen and mount in balsam. It is preferable to defer the cutting up of the mass until it is cleared, as otherwise the small blocks will sometimes curl.

With this method the specimen is surrounded by a tough, resistant matrix, and it is practically impossible to force an objective down on such a mount with sufficient force to cause the least injury to the object. Even if the cover is broken, the object is spared, and can easily and safely be remounted. However, the celloidin acts as a cushion, and ordinarily prevents the breaking of the cover unless the pressure upon it is very great.

This method has several other advantages. (1) The specimen may be transferred from the clearing fluid to the slide without any risk of injury. Hydra, for instance, can be thus mounted without the breaking off of buds or tentacles. (2) The use of rings, glass rods, or other cover-glass supports is entirely eliminated, for the celloidin not only prevents crushing of the specimen by pressure of the cover, but it also holds the cover level. (3) The specimen does not have a tendency to drift out of place when the cover is applied, as often happens if the usual method is used.

It is believed that this method will be found of considerable value in the preparation of slides for class use. Although it is often more tedious than the ordinary method of mounting, the insurance of the specimen against injury more than justifies the extra trouble.

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TRANSACTIONS
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Vol. XLII OCTOBER, 1923 No. 4

DESCRIPTIONS OF TWO SPECIMENS OF TWINNED EARTHWORMS OF WHICH ONE IS ADULT*

By
Frank Smith
University of Illinois

The first earthworm specimen described in this paper differs in some important characters from any others that have been seen by the writer, and from any adult specimens of which he has thus far found descriptions. It is unique among a rather extensive series of abnormal specimens which have been acquired in the course of a study of the earthworm fauna of the banks of a stream in the vicinity of Urbana, Illinois. Several thousands of earthworms have been collected, and more than fifty of them have shown external signs of abnormalities among the reproductive organs, but the specimen to be described is the only one which has shown evidence of a double or twin type of structure involving the anterior somites. That two individuals are represented is the obvious explanation for the eight pairs of setae on each of about 40 anterior somites; for the four oviducal and four spermiducal pores; and for the four distinct tubercula pubertatis, instead of the normal number of two. In the anterior two fifths of the length of the specimen this twin condition prevails, and then a rather abrupt reduction in the diameter is accompanied by a reduction of the setae to four pairs per somite; and the posterior three fifths of the specimen has the appearance of that of a normal individual.

A desire to know something of the internal organization of such a combination led to the preparation of transverse sections of the anterior 15 somites and of a few somites which include the posterior end of the smaller individual.

Preliminary to a more detailed description, a general statement of the relations of the two individuals represented and of some of the principal organs will be useful. The species represented is *Helodrilus tetraedrus* (Savigny). This is a highly variable species with several varieties or forms recognized by the systematists, but the specimen belongs to the typical form having spermiducal pores on the 13th somite, and anterior to the

*Contributions from the Zoological Laboratory of the University of Illinois, No. 227.

oviducal pores. Apparently two individuals of unequal size and number of somites are involved. The smaller one was fused throughout its length with the anterior part of the larger one, and but one mouth and alimentary tract functioned for both. The dorsal part of each individual, including the dorsal vessel, maintained its integrity and apparently the dorsal surface of the smaller specimen was next to the substratum and to that extent served as the ventral surface for the aggregate; but, the specimen having been killed before its peculiarities had been noted, nothing is positively known of its ordinary habits of locomotion nor which side was ordinarily next to the substratum. Since the length of the smaller individual was less than one half that of the larger, the arrangement suggested would result in having the ventral surface of the posterior half of the larger specimen in its normal relation to the substratum. There are two ventral nerve trunks, each with accompanying ventral and subneural blood vessels; and a line connecting the nerve trunks, in transverse sections of the specimen, would intersect at right angles a line connecting the two dorsal vessels, but at a point somewhat nearer to the dorsal vessel of the smaller individual. Each of these nerve trunks is apparently the result of the fusion of components belonging to both individuals, and instead of being in positions opposite to the dorsal vessels, they lie along what the casual observer would naturally consider as the lateral walls of the composite specimen. Figures 1, 2, 5, and 6 will aid in understanding these relations. For convenience the larger individual will be designated as A and the smaller one as B. Although each individual has its own dorsal parts intact, the right and left sides of its ventral parts are separated from each other and united with corresponding ventral parts of the other individual. The right ventral half of A is fused with the left ventral half of B and forms one of the two lateral surfaces of the composite specimen. Similarly the left ventral half of A is fused with the right ventral half of B and forms the other lateral surface. Corresponding relations prevail among internal organs related to the ventral parts, such as the reproductive organs and hearts. The relations are strikingly similar to those described by Vejdovský (1888-92) as existing in a juvenile specimen represented in figure 14, of plate XIX, in his paper, and described as "A) *Die Doppelmissbildung, wo die Individuen mit den Bauchflächen der ganzen Körperlänge nach verwachsen sind.*"

In the following description it will be assumed that the use of the expressions right, or left, side of the specimen refers to the side which includes the corresponding one of the A individual. A reference to the right side of the specimen will be to the one which includes the right side of A and the left side of B.

In addition to the characteristics of the specimen due to the double or twin structure, we find still further divergence from normal characters due

to irregularities in metamerism. Both the compound metamere and the spiral modification (Morgan 1895) are represented, and furthermore, in some instances, there are wide discrepancies between the external and internal indications of metamerism, such as intersegmental grooves, setae, and septa. The extremely close relationship between the individuals is well illustrated by the manner in which they are affected by these metameric irregularities. Wherever there is asymmetry among the organs and parts of one individual there is corresponding asymmetry in the closely associated parts of the other individual. The right side of A and the intimately associated left side of B differ in the same manner from the left side of A and the right side of B. Specific illustrations will appear in the details of the following description.

External Characters

It will somewhat simplify the description to adopt a system for designating the setae which is commonly used by systematists. It is customary to indicate the setae of either side by the use of the letters a b c d; the ventralmost seta being designated by a, the next by b, the next by c, and the dorsalmost one by d. The ventral pair includes a and b, and cd indicates a dorsal pair. The dorsal interval between dorsal pairs is d—d, and a—a is the interval between two ventral pairs. The description will be further simplified by adopting the custom of using arabic numerals to indicate the different somites and of numerical symbols for the septa and intersegmental grooves. The symbol 6/7 indicates either the septum or intersegmental groove separating the sixth and seventh somites. In the account of irregularities in metamerism, the term metamere will commonly be used instead of somite which is its equivalent in oligochaete terminology. This is done for the sake of conformity with the terms used by Morgan in his article describing such irregularities.

The specimen was killed moderately well extended and is 91 mm in length. The diameter varies from about 2½ mm in the genital region, and about 3 mm in the clitellar region, to about 1½ mm or less in the posterior half. The number of somites in A is about 114, and in B is a little over 40. Next posterior to intersegmental groove 7/8 there are two grooves encircling the entire trunk, giving the appearance of two complete somites to what is really a compound metamere or somite with two half metameres on the right side of the specimen and but one half metamere on the left side. That it is a compound metamere is shown by the presence of two full sets of setae on the right side and but one on the left side, and is also shown internally by the septal relations and the contained organs (fig. 2). Posterior to this compound metamere follows a single somite and then another compound metamere, as shown by the setae and internal organs, but surrounded by two complete grooves. This latter compound

metamere has the two half metameres on the left side and the single one on the right side. Up to this point, the number of somites indicated by the intersegmental grooves is one greater than the actual number, indicated by setae and internal organization. The second compound metamere is the beginning of a spiral modification, as shown by setae and by internal organization. Superficially, as shown by the grooves, the spiral begins with the groove 13/14, and extends posteriad five somites where normal segmentation is resumed. A half metamere is intercalated on the left side between somites 32 and 33 and a compound metamere with the two half metameres on the right side follows next posterior to the somite which includes 40 of the right side and 41 of the left. Somites posterior to 42 have normal numerical relations between their component half metameres.

In the specimen under consideration, the setal order for a somite, beginning with the dorsal side of A, is d c b a (of A); a b c d d c b a (of B); a b c d (of A); the aa setae being on the lateral surfaces, instead of on the ventral surface as in normal specimens. The setae first appear, as usual, on somite 2; four pairs or bundles in A, and but one in B. In 3, eight pairs are present, as in each of the somites next following; but the setae of B are very closely crowded. Eight is the usual number until somite 40 of the right side (41 of the left side) is reached, which has but six pairs. 41 of the right side is part of a compound metamere and has the dorsal and ventral pairs of the right side of A, and only the ventral pair of B. 42 of the right side is continuous with 42 of the left side, constituting the remainder of the compound metamere, and has six pairs. 43 has five pairs of which but one belongs to B. All of the succeeding somites have each but four pairs of setae, all belonging to A. The spacing of the setae in each pair is about normal for the species, but the dorsal interval d—d of B is only about one third as great as that of the same interval of A. This is the approximate ratio in the genital region and posterior to it. In a few anterior somites the discrepancy is still greater. In the fifth somite, d—d of A is nearly five times greater than in B. In somite 10, the setae of the ventral pairs are specially modified as genital setae. They are about twice as long as ordinary setae, rather slender, and are associated with glands which open into the setal apertures in the outer cellular layer in the body wall. Similar modified setae with like relations and location ordinarily occur in normal individuals. There is no apparent inequality in the size of the setae of the two individuals.

The clitellum is slightly asymmetrical which is probably due to the irregularities in metamerism. It includes ½ 22—27 on the right, and 23—⅓ 28 on the left side of A. Four well defined tubercula pubertatis are present, of which three are represented in figure 1. Those figured include the two belonging to B and the one on the right side of A. Two pairs of spermiducal pores are borne on prominent elevations of the body

wall on 13, and two pairs of inconspicuous oviducal pores are on 14. These pores have the same general relations to the setal bundles of the individuals A and B that one ordinarily finds in normal worms of this species, but the peculiar relations of A and B lead to the distribution of pores, from each of the two individuals, along each of the two lateral areas of the composite specimen. There is one spermathecal pore on the right side of A, in the groove 9/10; and two on the left side, in the grooves 9/10 and 10/11. There are none in B.

Internal Characters

There is no internal separation between the body cavities of the two individuals. The space between two consecutive septa is continuous between the two individuals to the same extent that it is between the two sides of either one of them. The coelomic spaces of B are much more restricted than are those of A and are nearly filled by the contained organs. Mention has already been made of certain internal irregularities. Following the seven anterior somites which are normal, a compound metamere results in having 8 and 9 of the right side of the specimen with a dividing septum, opposite to and in communication with 8 of the left side, which lacks the dividing septum. This is followed by 10 of the right side opposite 9 of the left side. Then follows a compound metamere or somite in which 10 and 11 of the left side are opposite 11 of the right side. Due to the introduction of a spiral modification in connection with this second compound metamere and extending posteriad for several somites, the spirally wound septum which begins anteriorly with the septum between 10 and 11 of the left side of the compound metamere allows free communication between the cavities of the constituent half metameres with each other, and with those following in the spiral (fig. 2). The general outcome of these complications is the normal location of most organs, if septa and setae are adopted as criteria for somite limits, as they are in this paper.

As previously stated, one alimentary tract functions for both individuals. It differs in structure from that of a normal worm and apparently includes at least the dorsal portions of the alimentary tracts of A and B. This is apparent from the structure and from the relations to the dorsal vessels. The normal condition in the fourth and fifth somites, in which there is a pouch formed by an evagination of the dorsal wall of the alimentary tract surrounded by an extensive mass of gland and muscle tissue, is replaced in the twinned specimen by such a pouch on each of the two sides of the pharyngeal lumen which correspond to the dorsal walls in both A and B. The one belonging to B is a little smaller than the other, but none the less typically developed. In normal specimens, a pair of evaginated pouches are formed in 10 from the latero-dorsal walls of the esophagus and are a part of the calciferous gland which is formed in the walls in 10

and a few following somites. Two normally formed pouches are present in A and are in the 10th somite, but the asymmetry due to irregularities in metamerism result in the pouch of the right side of A being formed anterior to the one on the left side. Instead of evaginated pouches, the wall of the alimentary tract in 10 of B is invaginated at the locations where pouches are normally formed. Instead of the normal typhlosole on the dorsal side, a typhlosole is developed on each of the two opposite walls of the intestine which are interpreted as being the dorsal parts of the two intestines of A and B united into a single tube. The typhlosole of B is not represented posteriad of the anterior part of the 44th somite.

The circulatory system has very interesting deviations from normal relations. A and B each has a dorsal vessel, and accompanying each nerve cord is a ventral and a subneural vessel. Each dorsal vessel with its branches presumably has its origin wholly from one individual, while each ventral and subneural vessel has probably had a double origin, as have also the vessels connected with them. The hearts, as in normal specimens, are paired in 7—11 of each individual and connect the dorsals with the ventral vessels (fig. 5). Hearts of the right side of A connect with the ventral vessel of the corresponding lateral side of the specimen, which also receives the hearts of the left side of B. Similarly the hearts of the left side of A connect with the ventral vessel of the left side of the specimen, as do also the hearts of the right side of B. Irregularity in metamerism has led to asymmetry in the relations of hearts on opposite sides of each of the dorsal vessels, but has not disturbed the paired relations between the hearts on the opposite sides of either of the ventral vessels. The large paired latero-longitudinal vessels which normally join the dorsal vessel in 12 are present in that somite in both A and B, one pair in each. The dorsal vessel of B extends to the anterior part of 44. The posterior end communicates with the vascular plexus in the intestinal wall through several large connecting vessels. The subneural vessels unite in 46, and the ventral vessels in the next following somite.

The nephridia are paired in most somites of each of the individual components of the specimen, and the locations of the nephridial funnels and external pores have normal relations in each. Posteriorly, the nephridia of B are not represented posterior to 45, and somites 43 to 45 have each but one nephridium, with pores very near the median line.

The study of the nervous system has been limited to an examination of the relations of the most important ganglia and ventral nerve cords. The supra-esophageal ganglion or "brain" of A has the normal position and gives rise to a pair of circumesophageal trunks of normal size, of which the one on the right side is continuous with one half of the ventral cord in the right side of the specimen, and the one on the left side is continuous with one half of the other ventral cord. Similarly the "brain"

of B, which is slightly smaller than that of A, is normally situated with reference to the pharyngeal wall of B and is connected by circumesophageal trunks with each of the ventral nerve cords (fig. 6). The trunk on the left side of B joins the one from the right side of A, each being continuous with that side of the ventral nerve cord which is related to the individual to which the trunk belongs. Similarly the circumesophageal trunk on the right side of B joins the one on the left side of A and each is continuous with a corresponding half of the ventral nerve cord on the left side of the specimen. Near the posterior part of B, the two ventral nerve cords approach each other, and in 44 and 45 there is a considerable decrease in the size of the half of each of them which belongs to the B individual. The first actual union of the two cords is in the anterior part of 46. An examination of sections of the cords, slightly anterior to the point of union disclosed the following facts concerning the relations of the three "giant fibres" in each of the cords anterior to the union, to those of the single cord posterior to the union. The outer giant fibre in each cord which was related to B disappeared; the middle and largest fibres of the two cords united into one; and the outer fibres in each cord which belonged to A was continued into the single cord, posterior to the union (figures 3 and 4).

The location of the principal reproductive organs in normal worms of the species under consideration is as follows: one pair each of testes and of spermiducal funnels in each of somites 10 and 11; one pair each of ovaries and of oviducal funnels in 13; one pair of sperm sacs in each of somites 9, (occasionally 10), 11, and 12; and one pair of spermathecae opening dorsally in close relation to each of the septa 9/10 and 10/11. The various reproductive organs of each individual of the twinned specimen are of normal size and location, with the exception of the spermathecae which are unrepresented in B and of which A has but three. There is no spermatheca in the right side of A, related to the septum and intersegmental groove 10/11. The various organs of the right side of A are distributed along one side of the nerve cord of that side of the specimen, and on the other side of the nerve cord are arranged the corresponding organs of the left side of B. The organs on the two sides of the nerve cord have a paired relation, but the constituent members of any pair belong to the different individuals A and B. Similarly the organs of the left side of A are associated with those of the right side of B, along the nerve trunk of the other side of the specimen. The irregularities in metamerism involve a correlated asymmetry among the organs on the two sides of either individual, but have not resulted in asymmetry in the arrangement of the organs on the two sides of the same nerve trunk. To illustrate: The testis and spermiducal funnel of the right side of somite 11 in A are opposite those belonging to the left side of somite 11 in B, but they are also opposite those of the left side of somite 10 in A.

The transition from the twinned condition to that of a single individual involves a somewhat gradual but continuous reduction in the body wall of B. This reduction begins with the disappearance of the mid-dorsal part of the wall and extends farther and farther laterad as one follows the sections posteriorly. In somite 40 (of the right side of the specimen), the dorsal pairs of setae of B and the part of the wall indicated by d—d have disappeared. In 43, the parts of the wall indicated by b—c have almost disappeared and the two longitudinal areas which include the ventral pairs of setae are closely approximated, and only one of these pairs develops in this somite. A gradual reduction of the remaining part of the wall a—a in the next two somites is followed by its complete disappearance in the anterior part of 46 where the union of the ventral nerve cords and accompanying trunks of the vascular system complete the transition from the twinned part of the specimen into that of the single individual.

General

In the foregoing description it has been assumed that the specimen studied includes two individuals united along their ventral surfaces. A discussion of this assumption involves references to a few publications dealing with the same general subject. Vejdovský (1888-1892) has contributed an extended account of the results of a study of twinning in several species of earthworms and an important discussion of the general subject. More recently Weber (1917), Welch (1921), and Newman (1923) have published in the same general field. Since some of these papers have bibliographic lists, it is unnecessary to introduce such lists in this paper. One species of earthworms which is very abundant in Europe and North America has been studied by several investigators and has been shown to produce, during early stages, large numbers of twinned specimens. This species has received various names in the literature on the subject, due to nomenclatural changes. *Lumbricus trapezoides*, *Allolobophora trapezoides*, and *Helodrilus caliginosus trapezoides* are simply different names for the same species, as has already been noted by Welch. Vejdovský found very many twinned specimens among the embryos and young obtained from cocoons of this species. He also found great variety in the mode or type of union which the twinned specimens exhibited. Of these various types, he gives chief attention to three. "Demnach werde ich im Nachfolgenden nur die charakteristischen Doppelembryonen anführen, solche nämlich, deren Verwachsungsflächen man ganz sicher ermitteln kann." "A) *Die Doppelmissbildung, wo die Individuen mit den Bauchflächen der ganzen Körperlänge nach verwachsen sind.* Ich fand mehreremals diese interessante Missbildung, immer je eins im Cocon. Die grösste war 8 mm. lang und ich habe sie auf der Taf. XIX, Fig. 14, nach dem Leben abgebildet." This figure is reproduced in figure 11 of this paper.

It has an appearance, at the posterior end, of dissimilarity in the size of the two individuals involved, and is very suggestive of what may have been the appearance of the specimen described in this paper, when it was at a similar stage of development. Vejdovský has figures of six transverse sections made from different parts of his specimen, which indicate the same plan of organization as that described in this paper. One alimentary tract representing the dorsal parts of the alimentary tracts of two individuals; each individual with its own brain, dorsal vessel, nephridia, and setae in normal relations. Two ventral nerve trunks, double in nature. "Es ist ersichtlich, dass jedes Bauchstrangsganglion einer Hälfte des einen und einer Hälfte des anderen Individuums seinen Ursprung verdankt."

In a more recent paper (Weber 1917), we have the results of an examination of the contents of 184 cocoons of North America representatives of the same species. Specimens were found representing various stages of development, from early cleavage to individuals with more than 100 somites. 35 specimens were twinned, and of these, twelve were sufficiently developed to have 60 or more somites in each individual. In six of the twelve, there was a dorsal union of the component individuals, but in no one of them did this union involve more than the anterior five somites. In none of the twelve specimens was there a union along the ventral surfaces and the author asserts the probability that the specimen described at length by Vejdovský, as an illustration of a union along the ventral surfaces, was really one in which the union was along the dorsal surfaces. She admits that the evidence for her view is not conclusive, since in Vejdovsky's specimen the individuals were united along their entire length; but the similarity of the relations between organs, as represented in Vejdovský's figures, to those shown by sections of her specimens in which she felt sure of dorsal surface union led her to assume that Vejdovský's interpretation was erroneous. She claims that the pharyngeal relations as shown in Vejdovský's figures support her views. The writer sees nothing in these figures that is contrary to what might be expected in the event of a union along ventral surfaces. The striking similarity of the adult specimen described in this paper, to Vejdovský's specimen make the same type of union for both highly probable. If the posterior three-fifths of the writer's specimen is really a part of but one of the individuals represented in the anterior twinned part, then the union must have been along the ventral surfaces. The claim that the union is along the dorsal surfaces involves the assumption that the posterior three-fifths of the specimen is made up of two parts, lying on either side of the sagittal plane, which have originated in two separate individuals, and that for some mysterious reason the other half of each has completely disappeared. There seems to be no basis for any such assumption and the writer believes

that his specimen belongs to Vejdovský's "A" type of twinned earthworms. The second type of union, as treated by Vejdovský, is: "B) Die Doppelmissbildung, *deren Individuen längs der Rückenseiten verwachsen*, fand ich nur in einem einzigen Falle." This type of union which was represented by but a single specimen among those studied by Vejdovský was found in six of the twelve specimens described by Weber. The third type is: "C) *Die polar verwachsenen Doppelmissbildungen* sind bei *Allolobophora trapezoides* die häufigsten, aber auch die Modificationen dieser Verwachsung sind sehr zahlreich." This type of union was found in three specimens of the twelve older ones in the Weber material.

The species in which Kleinenberg, Vejdovský, and Weber found a twinned condition among the young taken from cocoons to be of common occurrence is one of the most widely distributed and abundantly represented of any of the Lumbricidae of Europe and North America. Apparently very few, if any, survive from among the specimens in which the union involves any considerable number of anterior somites. No mention of such specimens in adult stages has been found by the writer, and no such specimens have been found by him or his assistants during an examination of several thousands of worms of this species. The specimen described in this paper belongs to the same genus, but to a species of which the early developmental stages have not been studied and reported, as far as is known to the writer. Apparently the twinned condition afforded no obstacle to success in the struggle for existence of this particular specimen, since it is one of the largest found among several hundred individuals of this species collected in this vicinity. Whether twinning occurs frequently, or rarely, among the embryos of this particular species is yet to be determined.

Welch in his recent paper on bifurcation of the embryos of Tubifex offers an explanation for the rarity of adult twinned specimens, even when the twinned condition is common among embryos. It is due to the almost insurmountable difficulties which twinned specimens encounter in their efforts to escape from the cocoons which are provided with openings barely large enough to allow the exit of normal specimens. He found also none surviving for any great length of time among the few twinned specimens that were released from the cocoons. For this latter fact no explanation is obvious.

The last few chapters of Newman's Physiology of Twinning (1923) include a discussion of symmetry reversal and mirror imaging. It is a subject which has received attention from a number of workers and one of which the explanation involves diversity of opinion. Normal earthworms are so thoroughly bilaterally symmetrical that they offer little opportunity for a study of symmetry reversal. The adult specimen described in this paper with its marked asymmetry between the two sides of either individual

furnishes an excellent illustration of symmetry reversal, and one which may be of interest to those striving to arrive at explanations of such phenomena. In this case the reversal involves organs which are in abnormal relations, and is obvious because of this abnormality. Presumably the special features of the abnormal relations are peculiar to the one generation and do not extend back through a long series of ancestors.

Twinning in *Sparganophilus Eiseni*

Welch in referring to the literature on bifurcation among Oligochaeta, makes this statement concerning embryonic bifurcation: "All recorded instances fall within the Lumbricidae." In his paper he has shown that it also occurs within the family Tubificidae. A recent paper (Hague, 1923) has made known that this phenomenon is found occasionally in *Sparganophilus eiseni* which belongs to the family Glossoscolecidae, or, if we follow Michaelsen (1921), to a more restricted family Sparganophilidae. Four juvenile specimens of this species which were taken from cocoons are bifurcate posteriorly and exhibit a dorsal type of union, anteriorly, of 7—25 somites. One of these specimens which had been collected at Douglas Lake in Cheboygan County, Michigan, was presented by Dr. Hague to the writer and from it figure 10 was prepared, showing some of the characters recognizable in it as an unstained specimen in cedar oil. The anterior 15 somites of the two individuals were united and the two separate posterior parts each contained about 25 well defined somites and a terminal growing region. Transverse sections of the anterior twinned part show the presence in each component individual of a mouth and alimentary tract in the first five somites. In the next few somites these are united into a single esophageal tube. Posterior to the 14th somite the alimentary tracts are again separate and extend into the two posterior trunks. In close contact with each other and between the two alimentary tracts, lie the supraesophageal ganglia of the two component individuals (fig. 7, 8, and 9, br.) and each is connected by circumesophageal trunks with the corresponding ventral subesophageal ganglion. There is no separation between the coelomic cavities of the two individuals in the anterior twinned part. Nothing has been found of a well defined circulatory system, but traces of what appear to be the beginnings of dorsal vessels are found on either side of the alimentary canal and about equidistant from the two ventral nerve cords.

Summary

A description is given of the anatomy of a twinned specimen of *Helodrilus tetraedrus* which had attained sexual maturity.

The general organization is similar to what might be expected if one should succeed in splitting the anterior 45 somites of a normal individual

in a sagittal plane from the midventral surface as far as the middle of the lumen of the alimentary tract; then in spreading the cut surfaces apart and inserting a specimen similarly treated which had a corresponding length and number of somites (placing the cut surfaces in contact), thus producing a specimen with a double number of setae, reproductive organs, nerve and vascular trunks and nephridia, but with a single alimentary tract and mouth.

The double or twinned condition prevails in the anterior 45 somites and is followed by about 70 somites of the type of organization found in the posterior part of a normal individual.

Certain irregularities in metamerism with consequent asymmetry in some of the reproductive and associated organs are described.

A brief description is given of a juvenile twinned specimen of *Sparganophilus eiseni*, in which there is a dorsal type of union of the anterior 15 somites of the two individuals, followed by separate posterior parts of about 25 somites each.

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Plate 1

Explanation of figures

1. Showing the dorsal part of B, in the posterior half of the twinned part of the specimen. The lower part of the figure and a few somites at the right are from A, as seen from its ventral side. *Cl*, clitellum; t p B and t p A, tubercula pubertatis of B and A, respectively. 20, 30, and 40, numbers of somites on left side of specimen.

2. Diagram showing the relations of parts and organs in somites containing the reproductive bodies, represented as though the body wall had been split along the median dorsal surface of A and then spread out and viewed from the inner surface. a, b, c, and d, setae; d v, dorsal vessel; n c, nerve cord; A(1) and A(r), left and right halves, respectively, of A; 7–14, numbers of somites; ♀, oviducal pore; ♂, spermiducal pore; o, ovary; s, testis or spermary; o f, oviducal funnel; s f, spermiducal funnel; s s, sperm sac; s t, spermatheca; sep, septum.

3. From transverse section near posterior end of B, showing union of the two ventral nerve cords and near approach of the two ventral vessels and the two subneural vessels. m f, median giant nerve fibre; n c, nerve cord; s v, subneural vessel; v v, ventral vessel.

4. From a section slightly posterior to one shown in figure 3, showing closer approach of parts. Same abbreviations as in figure 3.

5. Diagram showing relations of "hearts" and longitudinal blood vessels; d v, dorsal vessel; h, heart; n c, nerve cord; v v, ventral vessel.

6. Diagram showing relations of brain, circumesophageal nerve trunk, and subesophageal ganglion at anterior end of ventral nerve cord. br, brain; c t, circumesophageal trunk; s g, subesophageal ganglion.

Plate 2

Explanation of figures

7, 8, and 9. Oblique sections through anterior somites of specimen figured in 10. Obliquity such that the part shown on the right side of each figure is approximately two somites posterior to that shown on the opposite side. c d, fragments of setae of dorsal pair.

7. From section through mouth opening on one side, and setae of third somite on opposite side. a t, alimentary tract; br, one side of brain; m, mouth; c d, dorsal setae of third somite.

8. From section through region about one somite posterior to that shown in figure 7, b r, brain; s g, subesophageal ganglion; c d, dorsal setae of fourth somite.

9. From section through region about two somites posterior to that shown in figure 7. a t, alimentary tract; b r, brain; c t, circumesophageal trunk; s g, subesophageal ganglion; c d (at left side), dorsal setae of second somite; c d (at right side), dorsal setae of fifth somite.

10. From anterior part of twinned juvenile specimen of *Sparganophilus eiseni*, with dorsal type of union. b r, brain; m, mouth; n c, nerve cord; p, prostomium (combined).

11. From figure 14, plate XIX, Vejdovský (1888–1892). Showing twinned juvenile earthworm with ventral type of union.

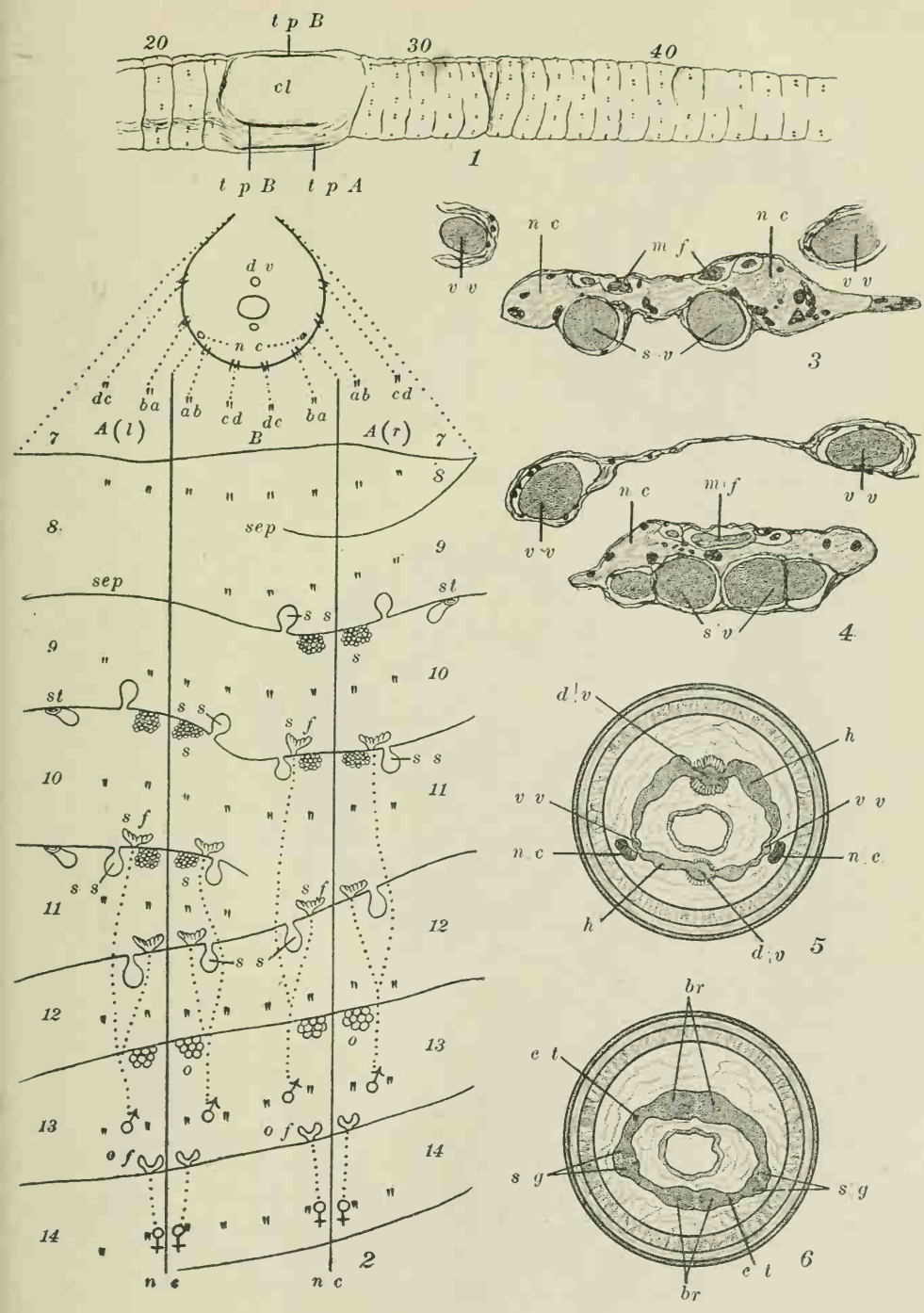
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PLATE I

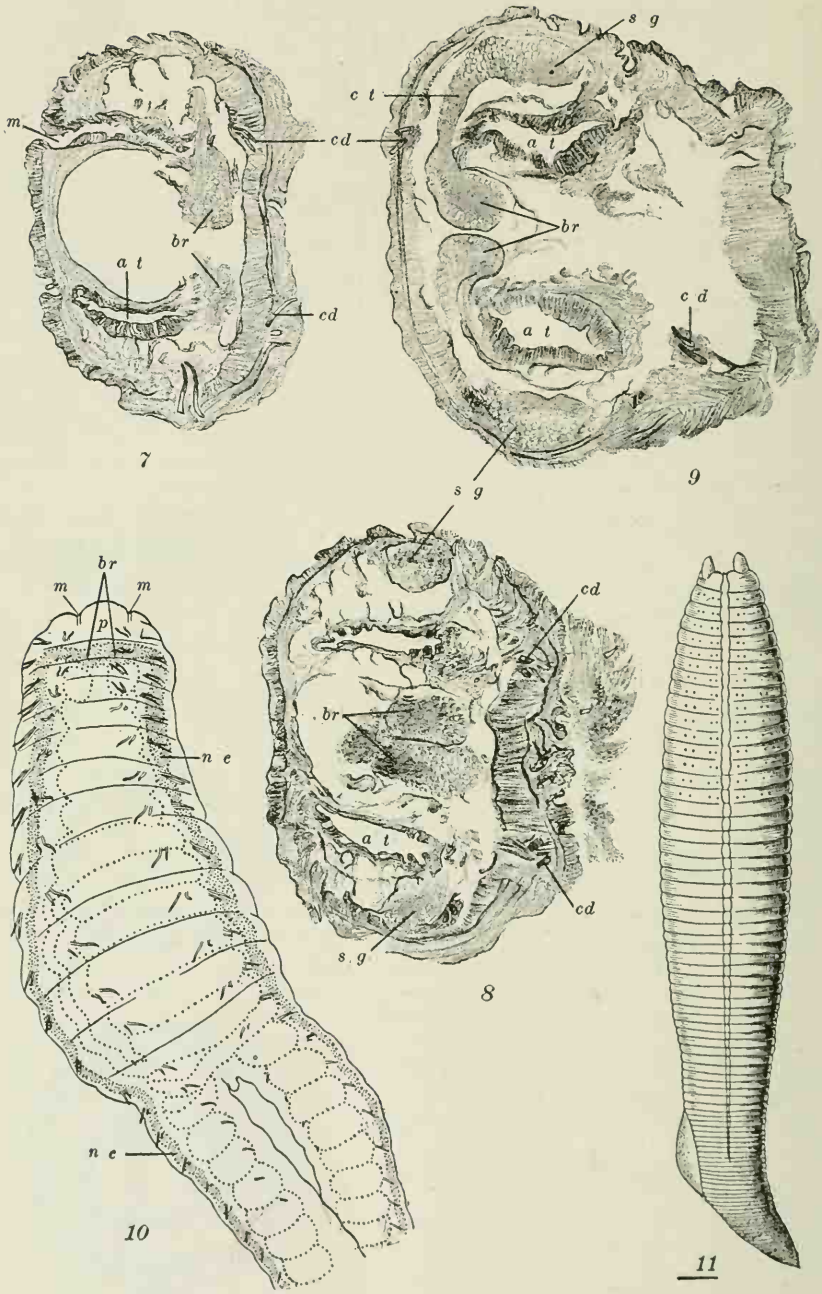

PLATE II

A REVISION OF THE DESCRIPTION OF *DIPLOCARDIA MICHAELSENI* EISEN*

By
Frank Smith
University of Illinois

Eisen's preliminary description of this species appeared in 1899 and a more extended account in 1900. Each of these papers contained a review of the various species of Diplocardia then known, and a suggestion of the probable existence of a still greater number of unknown species. The description of *D. michaelseni* was based on material from Raleigh, N. C., collected by Messrs. Brimley, and Eisen states that he had a dozen specimens. In 1899, the year in which the preliminary description was published, Dr. Eisen sent to the writer several specimens of earthworms of different species, one of which was accompanied by a label which reads "Diplocardia michaelseni Eisen. Raleigh, N. C. April, 1898. Eisen det." In the absence of any evidence to the contrary it seems natural to assume that this may be one of the dozen specimens which Eisen mentions. In the preparation of a paper dealing with new and known species of Diplocardia, the writer, being desirous of certain data concerning Eisen's species which were not supplied by his papers, has prepared and studied sections of some of the specimens which Dr. Eisen had sent him. A result of this examination has been the discovery of a few discrepancies of some importance between the description of *D. michaelseni* and the specimen supplied, which will receive attention. Eisen states that only one of his specimens of this species was sectioned. The writer has studied sagittal sections of one half of the anterior 23 somites of his specimen.

It will simplify the comparison between Eisen's description of the species and the results of this examination if we first consider characters in which there is agreement. We have the following: "Prostomium divides somite 1 completely." "Setae all ventral; a—a = 3 a—b; a—a about one third larger than b—c." Eisen makes no mention of the distance c—d which in the writer's specimen is about 1½ times a—b, and a—b is slightly less than described by Eisen. Clitellum on 13—17 and encroaches somewhat on the dorsal part of 18, and is but slightly developed ventrally on 13. A "spermathecal genital zone" includes pairs of large rosette-like papillae on the ventral side of 8 and 9. Setae a and b of these two somites are highly modified and sculptured (Eisen, 1899, fig. 2). A "post-clitellar genital zone" includes a median ventral depression on somites 18—20,

*Contributions from the Zoological Laboratory of the University of Illinois, No. 228.

connected anteriorly with two deep pits in the posterior margin of 17. Somites 21-23 have on the ventral side two pear-shaped swellings. Only the anterior one is well defined in the writer's specimen. Grooves connecting the prostate gland pores of either side are approximately straight. These pores are paired on 18 and 20 and the spermiducal pores on 19. In the writer's specimen, the latter pores are near the anterior margin of the somite. Oviducal pores on 14 are near the anterior margin and very close to the mid-ventral line and to each other. The spermathecal pores have relations which distinguish the species from the other known species of the genus. There is one pair on 8, slightly anterior to the ventral pairs of setae and each about equally distant from a and b of the same side. A second pair is present on 9, but each opens near the posterior margin of the somite, nearly in seta line a.

Septa 7/8 and 8/9 are more strongly thickened than others. Spermaries, spermiducal funnels, ovaries, and oviducal funnels have the usual number, positions, and relations. There are paired sperm sacs in 9 and 12. Two pairs of prostate glands occupy a good deal of the available space in somites 17 or 18 to 21 inclusive. The muscular ducts are long and contorted and open on 18 and 20. The glandular parts have a peculiar structure described in detail by Eisen. The lumen of each is very inconspicuous, without definite epithelial lining, and of no greater diameter than that of the duct. Numerous branches of the lumen are formed and each is surrounded by a single layer of gland cells. Spermathecae are paired in 8 and 9, and have long contorted ducts, each of which has a long diverticulum, directed anteriorly, and a definite sac-like expansion which has a length greater than the diameter of the worm, and a strong constriction about midway of its length. Bundles of glands, developed in the body wall, extend parallel with the long axis of the body and open in close relation with the apertures of the modified spermathecal setae. The development of these glands is largely responsible for the papillae on the ventral surfaces of 8 and 9.

We have now to consider several features in which the specimen received from Dr. Eisen differs from the description of *D. michaelseni*. In the description we find: "Size 45 mm. by 2 mm., hardly tapering posteriorly. Somites 63." The writer's specimen is about 65 mm. by 1½ mm. and has 116 somites. Presumably the type specimen on which the description was based had lost a number of somites from the posterior end. In his first paper Eisen says: "Penial setae present at spermiducal pore" and makes no mention of such setae at the prostate pores. In his second paper the "Definition" which is chiefly a repetition of the matter in the preliminary paper has the same reference to penial setae, while in the more extended description we find no reference to the setae at the spermiducal pore, but a statement that the prostates "open as usual near sacs with

penial setae, but I am unable to say whether these are sculptured or smooth." The writer has found no setae at the spermiducal pores in his specimen. The penial setae at the prostate pores are very inconspicuous and but little could be learned of their characters in the relatively thick sections containing them. As indicated above, there is general agreement in the positions of the various reproductive organs.

Eisen states that "The enlargement of the septa is principally dorsal," referring especially to 7/8 and 8/9. His figures indicate a specimen nearly straight and not strongly contracted anteriorly. Figure 1 of this paper shows the anterior part of the writer's specimen strongly contracted anteriorly and curved ventrally which may easily account for the fact that the septa 7/8 and 8/9 are thickest ventrally. The same figure shows clearly

FIG. 1. Combination of parts of several sections with the aid of a camera lucida, showing relations of the body wall, septa, and alimentary tract. g, gizzard; s s, distal part of setal sac; st po, spermathecal pore; 5/6, septum between somites 5 and 6.

that the gizzards are in somites 5 and 6, and not in 6 and 7, as stated in the description. Eisen makes special reference to a glandular crop in 14 and 15 and states: "As far as we know it also differs in the possession of a glandular crop in two of the clitellar somites, similar to the one described in *Pontodrilus michaelseni*." Eisen overlooked the similarity of this organ to the one in the same situation in *Diplocardia eiseni* Michaelsen which had been described in detail by Michaelsen in 1894, and which is even somewhat more highly differentiated, and has more numerous and strongly developed folds. Figure 2 shows something of the general character of this organ in *D. michaelseni*, as represented in the writer's specimen. Its position was somewhat oblique and sections that are nearly median in 15 are more nearly tangential in the part lying in 14. There is much evidence in support of Michaelsen's supposition that this organ is of the nature of a calciferous gland.

In the description, Eisen states: "I find muscular connecting vessels or hearts in 10 and 11 only. There is no supra-intestinal dilatation of the dorsal vessel, as in some other species. The dorsal vessel appears to be single." In the writer's specimen, there is an additional pair of

hearts in the twelfth somite which are fully as large as those of 10 and 11. The part sectioned did not extend quite far enough to permit a positive statement, but the indications are that the hearts of 11 and 12, at least, are dorso-intestinal hearts, by which is meant that they have their larger dorsal opening into a supra-intestinal trunk, and a smaller connecting vessel opening into the dorsal vessel. The supra-intestinal trunk has its origin and termination in the vascular plexus of the wall of the alimentary tract and is not a part of the dorsal vessel as implied in Eisen's statement. In the description, it is stated that sperm sacs are present in 9, 10, and 12; those of 10 being postseptal. This would imply a communication of those in 10 through the septum into 9 in which there are no spermaries nor sperm ducts. In the writer's specimen, there are no well defined sperm sacs in 10 and there is but little difference in appearance between the masses of sperm cells in 10 and 11.

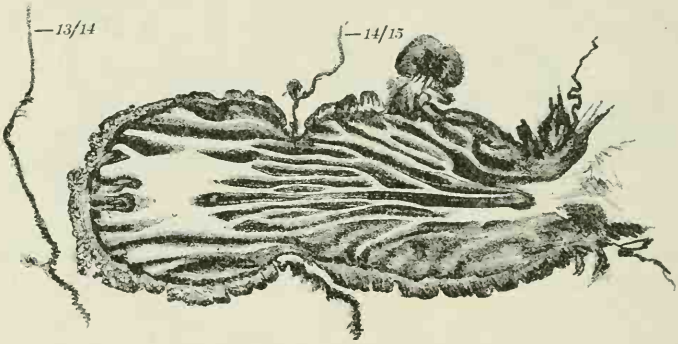

Fig. 2. From a sagittal section through the alimentary tract in somites 14 and 15. Section nearly median in posterior part of caliciferous gland and more nearly tangential in anterior part. 13/14 and 14/15, septa between 13 and 14, and between 14 and 15.

In spite of the few discrepancies between Eisen's description and the details of structure exhibited by the specimen, the writer is convinced that the specimen really belongs to the species to which it was assigned by Eisen, and that the discrepancies are due in part to oversight and errors of observation in the preparation of the original description, and perhaps in part to individual differences in specimens. Unfortunately the destruction of Eisen's collections at the time of the San Francisco earthquake has presumably made another examination of the type specimen impossible. The septa related to the gizzards are quite thin and difficult to follow in sections, and, if not considered, the gizzards are very likely to be allotted to somites posterior to the ones to which they actually belong, since they are commonly found shoved posteriad and lying under divisions of the body wall which are a somite or two posterior to the ones to which they are actually related. This is illustrated in figure 1 which represents the

septa as somewhat more distinct than they actually are. This apparent dislocation posteriad is common in Diplocardia. Such a condition is perhaps related to the frequent eversion of the buccal cavity in normal feeding activities, which must involve a good deal of flexibility and freedom of movement in the anterior parts of the alimentary tract. It is difficult to account for the statement that hearts were found in 10 and 11 only, since no Diplocardia species or specimens are known to the writer, in which the hearts are not present at least as far posteriad as the twelfth somite.

The following characterization of the species includes the few changes made necessary and is believed by the writer to be more accurate than the original description.

Diplocardia michaelseni Eisen

Length, 45—65 mm. Somites, 63—116. Clitellum, 13—½18, but little developed on ventral side of 13, cingulum. Papillae on 8 and 9 in close relation to ventral setae and to glands developed in the body wall; one or two pear-shaped swellings on ventral side of 18—20. Spermiducal pores, paired on 19; prostate gland pores, paired on 18 and 20, those of either side connected by nearly straight longitudinal grooves. Oviducal pores, paired near anterior margin of 14 and near mid-ventral line. Spermathecal pores, paired on 8, slightly anterior to ventral setae; and paired on 9, but near posterior margin of that somite and nearly in seta line a. aa:ab:bc:cd = 6:2:4:3. Ventral setae, of 8 and 9, highly modified as genital setae; none on 19. Septa, 7/8 and 8/9 most heavily thickened. Gizzards, in 5 and 6. Calciferous gland, with conspicuous longitudinal folds, in 14 and 15. Dorsal vessel, double. Hearts, paired in 10—12. Spermaries and spermiducal funnels, paired in 10 and 11. Sperm sacs, paired in 9 and 12. Prostate glands, two pairs, 17—21. Ovaries and oviducal funnels, paired in 13. Spermathecae, paired in 8 and 9; each with one long diverticulum.

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RECENT WORK ON MARINE MICRO-PLANKTON AT THE LA JOLLA BIOLOGICAL STATION

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In the earlier years of the Scripps Institution for Biological Research and of the San Diego Marine Biological Association which preceded it, all efforts at investigations were necessarily devoted to surveying the field in quest of most promising avenues of research and to laying a foundation of taxonomy, general morphology, general habitat range and general distribution studies. In 1919 it was decided that sufficient progress had been made in basic studies and in physical oceanography to warrant an effort at more intensive work on diatoms and dinoflagellates, in addition to some already in progress with the copepods and chaetognaths.

Local experience as well as authoritative literature had shown that ordinary methods of collecting microplankton required a great deal of skill, expensive equipment, considerable time and space and a number of favorable conditions for operation. Furthermore, it was well known that such methods could not yield very accurate results in estimating the plankton population of the area sampled.

After special experiment the most satisfactory method was found to be that of dipping up water in some kind of container and pouring it in measured quantity through a small silk net or other filtration device. Number twenty-five bolting silk was adopted as the standard filtering material for diatoms and for other forms of similar size. For diatoms twenty-five liters was found to be satisfactory as a standard quantity of water. On account of accessibility the surface was arbitrarily selected as the standard level and fixed inshore stations (such as piers) as standard stations for collecting microplankton.

As a result of such methods and plans of operation the range and continuity of collecting has been greatly extended. An unskilled workman of careful habit can make just as good standard collections as can an expert. Initial equipment for standard work costs only a small fraction of the former ordinary expenditure in starting plankton work and the operating expense is reduced far more since frequent replacement is no longer required. The saving of time and space in making standard collections is also very great. In addition we have convincingly shown that valuable series of collections can be taken under conditions formerly prohibitive. It is also evident that statistical treatment of material

obtained by standard methods yields estimates of population which are more accurate than it is physically possible for the sampling to be. And, of course, the continuity of series possible by such standard methods strongly augments the value of the statistical records.

At our own pier twenty-five liter collections have been made daily for a little more than four years, at Pt. Hueneme (except Sundays and holidays) for nearly four years, and similarly at Oceanside for nearly three years. These have been supplemented by fragmentary and short series at Redondo, Santa Barbara and Pacific Grove. Such continuous series could not have been obtained by us from even these few localities by any other method.

At no time have we intended to confine ourselves to standard methods and localities if resources and opportunity should make other methods possible or should admit us to other localities. In fact, the excellence of the general method has been shown more strongly in some supplementary series taken by us than it has been in the standard series.

One series was taken in 1920 from Jacksonville, Florida to San Diego. This was broken by rough weather in the Caribean Sea, but Crandall and Michael, our most experienced plankton men, did the work and they both emphatically stated that on that trip no series at all could have been taken by any other method. Not long afterward a short series followed by occasional catches over a period of six months was taken between San Diego and Seattle from the Pacific Steamship Company's passenger steamer Queen. None of these catches could have been obtained by any other method. In both of these cases only three gallons of water was filtered but even that small amount gave good results.

For the last three summers we have chartered a tuna fishing boat for a few weeks work at two stations respectively five and ten miles seaward from our pier. By use of the Kofoid closing bucket which holds five gallons of water we have been able at these two stations to get for a time daily catches at the surface and at twenty and forty meter levels. These series differed from standard series only in the fact that samples were taken from lower levels as well as from the standard surface level. All alike were filtered through the standard net and handled in the standard way. The marine experts of our staff are agreed that by no other method than the dipping method could such satisfactory catches be so continuously made or with so little equipment or at such small expense. For larger plankton and for chemical examination other catches were made at these stations with ordinary tow nets and closing nets.

In 1921 the California Academy of Sciences conducted some investigations in the Gulf of California in connection with which a series of diatom collections was made by the dipping method. A somewhat similar series was obtained in 1922 in Pacific waters off Lower California through

the courtesy of the Mexican Government which was conducting a biological survey of Pacific Islands. In the latter part of January 1923 the U. S. Coast and Geodetic Survey steamer Pioneer began taking collections for us which were continued from San Diego to Alaska. In none of these cases would series of collections have been possible by customary methods. Yet they are giving very excellent results.

Beginning with July 1922 a series of collections of zooplankton was begun by the dipping method with filtration through the same net as was used for diatoms. On account of smaller numbers of animals it was necessary to filter one hundred liters of water instead of twenty-five. This material was examined alive for identification and the contents counted or estimated after killing by Dr. Helen E. Murphy. So far as can be judged at present the results are just as promising as those for diatom material. This is especially true concerning the larval forms which have such great numerical prominence in the zooplankton.

The taxonomic and statistical work on marine copepods which Dr. Esterly has been doing for several years has reached a temporary climax in his recent publication of studies indicating seasonal distribution of certain representative groups and in present efforts to segregate several small species which have numerical importance but which are very difficult to recognize under the low magnification necessary to counting.

The migratory habits of the copepods and some other plankton animals indicate that they are more favorable subjects for statistical studies of short period correlations than are the diatoms, and it seems probable that the work done by Dr. Esterly constitutes a valuable foundation for statistical studies outlined by Dr. McEwen. Under such conditions the prospect for advancement in copepod studies is limited only by the resources and facilities which can be applied to such work.

In the last four years Dr. Essenberg has done some interesting work on taxonomy and distribution of the Appendicularia, but present indications are that that group is not sufficiently prominent in our plankton population to warrant continued special attention with our present limits of facilities.

The results of our various methods of work in these somewhat different but closely related lines have been very satisfactory. The most conspicuous features of these results are as follows:

1. All microplankton organisms show marked seasonal variations in numbers. In higher levels of the sea Appendicularia are rare in the warmer and sometimes very abundant in the colder months. Copepods show larger numbers of adults in Spring and mid Summer with marked abundance of particular species at other times. Diatoms are usually few in mid and late Summer, most abundant in Spring, next most abundant in Fall and sometimes prominent in late Winter. Dinoflagellates tend to

be most numerous in Spring, in late Summer and in Fall with some cases of remarkable production in late summer.

2. Many zooplankton organisms, especially copepods, are strongly migratory and usually negatively heliotropic. Hence, larger catches are to be expected at or near the surface at night rather than in the day time. There is evidence that tropisms may be reversed in response to changes such as that of salinity but definite field work along that line is still lacking.

3. There is rapidly increasing evidence that the microplankton population is unevenly distributed in most areas, both as to total numbers and as to proportional representation of various species.

4. Present indications are that prominent plankton diatoms of one oceanic area are likely to be found prominent in any area between the Gulf of California and Puget Sound. Furthermore, it appears that most of the important forms observed in Pacific waters are likely to be found at some time of year in any part of this general region.

5. We have some evidence that heavy production of pelagic diatoms is (south of Point Conception) characteristic of the sea rather than of bays and lagoons.

6. Although heavy production of diatoms has been found nearly one hundred miles from shore and near the limits of the continental shelf there is good ground for thinking that the most productive areas, especially below Pt. Conception, are within fifteen or twenty miles of shore, possibly less.

7. We have considerable evidence that the most productive level for diatoms is between ten and twenty meters below the surface.

8. Although surface levels are somewhat less productive than slightly lower levels there is reason for thinking that general responses to environment are similar and that the trend of seasonal distribution is the same.

9. Conditions which cause heavy production of one or two species of diatoms or dinoflagellates usually cause slightly increased production of other species although these may be less conspicuous by comparison. Furthermore, such favorable conditions also cause an increase in the total number of species listed.

10. Although it is assumed that changes in temperature must have a marked influence on all plankton organisms it has been found that heavy production of diatoms may be very conspicuous at the same time at all levels from the surface to thirty meters below in ranges of temperature similar to those from December to June at the surface.

11. Our practical experience and our experiments have led to the adoption of methods and plans for work on phytoplankton which seem to be as satisfactory as the nature of the work will permit.

A KEY TO THE GENERA OF ACANTHOCEPHALA*

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More than fifty generic names have been proposed for members of the relatively small group of parasitic worms known as the Acanthocephala or thorny-headed worms. Not all of these names are valid but there is no compilation or analysis of these names which is of service to the general worker in parasitology. Stiles and Hassall have made most valuable contributions (1920) in their Index-Catalogue to the Roundworms but this work, which is excellent for references prior to 1915, stops short of the period in which the most active work has been in progress. Seventeen of the generic names in the following list are more recent than the Stiles and Hassall catalogue.

There have been continuous additions to the knowledge of the members of this group since the time of the pioneer workers in parasitology dating from the latter part of the eighteenth century but the systematic organization of this information had its beginning only about three decades ago. Prior to 1892, all species of Acanthocephala were, by most workers, placed within the single genus Echinorhynchus. Beginning with Hamann's contributions of that year, the number of generic names has increased at a very rapid pace. No attempt has ever been made to prepare a key which would make it possible for anyone other than a specialist, to recognize the genera of specimens encountered in general collecting. A number of workers have expressed the desirablity of having such a key and in the belief that it would be of value the present one has been formulated. The involved synonymy and frequent confusion of generic names has made it seem desirable to preface the key by a tabulation of some of the more important data concerning each genus.

In differentiating the genera of Acanthocephala, it is not always possible to utilize external characters which are observable in living or preserved specimens. Usually the specimens must be prepared for microscopic examination. In following the appended key, well prepared toto-mounts or sections are usually essential. In the preparation of permanent mounts, many catastrophes and disappointments are avoided by puncturing the body wall of each specimen with a dissecting needle while the specimens are still in the preservative. Stains and the mounting medium enter the body cavity and the internal organs much more readily in punctured

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TABLE I.

The Genera of Acanthocephala.

	generic name	author	date	type species	Pisces	Amphib.	Reptil.	Aves	Mammalia	distribution	status
1.	Acanthocephalus	Koelreuter	1771	anguillae	x	x				Holarctic	valid
2.	(Acanthrus)[1]	Acharius	1780	sipunculoides	x					Palaearctic	s.o. Echinorhynchus
3.	Apororhynchus	Shipley	1899	hemignathi				x		Australian	valid genus
4.	(Arhynchus)	Shipley	1896	hemignathi				x		Australian	s.o. Apororhynchus
5.	Arhythmorhynchus	Lühe	1911	frassoni				x		Holarctic	valid
6.	(Bolborhynchus)	Porta	1906	?					x	Holarctic	s.o. Bolbosoma
7.	Bolbosoma	Porta	1908	?					x	Holarctic	valid
8.	Centrorhynchus	Lühe	1911	aluconis				x		Holarctic	valid
9.	(Chentrosoma)	Monticelli	1905	?				x		Holarctic	in part s.o. Corynosoma
10.	Corynosoma	Lühe	1904	strumosum				x	x	Holarctic and Neotropical	valid
11.	(Echinogaster)[2]	Monticelli	1905	sagittifer	x					Nearctic (plus?)	s.o. Serrasentis, nom. nov.
12.	Echinopardalis	Travassos	1918	pardalis					x	Neotropical	valid
13.	Echinorhynchus	Zoega in Müll.	1776	gadi	x					Holarctic	valid
14.	(Echinosoma)	Porta	1907	?	x					Palaearctic	homonym
15.	(Empodius)	Travassos	1917	empodius				x		Holarctic	s.o. Mediorhynchus
16.	(Eorhynchus)	VanCleave	1914	rutili	x		x			Holarctic	s.o. Neoechinorhynchus
17.	Filicollis	Lühe	1911	anatis				x		Cosmopolitan?	valid
18.	Gigantorhynchus	Hamann	1892	echinodiscus					x	Neotropical	valid
19.	Gracilisentis	VanCleave	1919	gracilisentis	x					Nearctic	valid
20.	(Haeruca)	Gmelin	1790	muris					x	Palaearctic	a larval cestode
21.	(Haerucula)	Pallas	1760	?	x	x				Palaearctic	s.o. Echinorhynchus
22.	(Hamannia)	Travassos	1915	microcephala					x	Neotropical	s.o. Hamanniella
23.	Hamanniella	Travassos	1915	microcephala					x	Neotropical	valid
24.	(Heteroplus)	Kostylev	1914	otidis				x		Holarctic, (Neotrop.?)	s.o. Mediorhynchus
25.	(Hormorhynchus)	Ward	1917	moniliformis					x	Cosmopolitan	s.o. Moniliformis
26.	(Koleops)	Lockwood	1872	anguillae	x					Nearctic	unrecognizable
27.	(Lepidosoma)	Porta	1907	lamelliger	x					Australian	homonym. s.o.? Serrasentis
28.	Lüheia[3]	Travassos	1919?	lühei				?		Neotropical	uncertain
29.	Macracanthorhynchus	Travassos	1917	hirudinaceus					x	Cosmopolitan	valid
30.	Mediorhynchus	Van Cleave	1916	papillosus				x		Holarctic, (Neotrop.?)	valid
31.	Micracanthorhynchus	Travassos	1917	emberizae				x		Neotropical	validity questioned
32.	Moniliformis	Travassos	1915	moniliformis					x	Cosmopolitan	valid

TABLE I. (continued)

The Genera of Acanthocephala.										
generic name	*author*	*date*	*type species*	*Pisces*	*Amphib.*	*Reptil.*	*Aves*	*Mammalia*	*distribution*	*status*
33. Neoechinorhynchus	Hamann in Stiles and Hassall	1905	rutili	x		x			Holarctic	valid
34. (Neorhynchus)	Hamann	1892	rutili	x		x			Holarctic	homonym. s.o. Neoechinorhynchus
35. Octospinifer	Van Cleave	1919	macilentus	x					Palaearctic	valid
36. Oligacanthorhynchus	Travassos	1915	spira				x		Neotropical	valid
37. Oligoterorhynchus	Monticelli	1915	campylurus				x		Palaearctic	probably valid
38. Oncicola	Travassos	1917	oncicola					x	Neotropical	valid
39. Pandosentis	Van Cleave	1920	iracundus	x					Neotropical	valid
40. (Paradoxites)	Lindemann	1865	renardi				x		Palaearctic	homonym. s.o. Centrorhynchus?
41. (Pardalis)	Travassos	1917	pardalis					x	Neotropical	homonym. s.o. Echinopardalis
42. Plagiorhynchus	Lühe	1911	lanceolatus				x		Holarctic	valid
43. Polymorphus	Lühe	1911	minutus				x		Cosmopolitan?	valid
44. Pomphorhynchus	Monticelli	1905	laevis	x					Holarctic	valid
45. (Proboscidea)	Bruguière	1791	?						?	s.o. Echinorhynchus sensu latu.
46. Prosthenorchis	Travassos	1915	elegans					x	Neotropical	valid
47. Prosthorhynchus	Kostylev	1916	?				x		Palaearctic	valid
48. (Pseudoechinorhynchus)	Goeza	1782						x	Palaearctic	a cysticercus
49. Quadrigyrus	Van Cleave	1920	torquatus	x					Neotropical	valid
50. Rhadinorhynchus	Lühe	1911	pristis	x					Holarctic (plus)	valid
51. Serrasentis[2]	Van Cleave	1923	sagittifer	x					Nearctic (Australian?)	nom. nov. for Echinogaster
52. Tanaorhamphus	Ward	1917	longirostris	x					Nearctic	valid
53. Tegorhynchus	Van Cleave	1920	brevis	x					Neotropical	valid
54. Telosentis	Van Cleave	1923	molini	x					Nearctic	valid

[1] Generic names in parenthesis are not valid.

[2] Serrasentis, nom. nov. for Echinogaster, Monticelli, Preoccupied. For full generic diagnosis see genud Echinogaster in Jour. Parasitol., 5:22.

[3] Travassos (1920) lists this name but the writer has never seen a diagnosis of the genus.

specimens than in those with intact cuticula. Balsam or damar mounts of specimens stained in dilute hematoxylin, especially Ehrlich's acid hematoxylin, are best suited for study. In some of the larger specimens, which are too large for examination with the compound microscope, dissections are essential. Synthetic oil of wintergreen as a clearing agent renders even fairly large specimens remarkably translucent. Whole mounts cleared in this oil and mounted in damar usually show all characters needed for the identification of genera and often are sufficient for the determination of species.

The accompanying figure (Fig. 1) shows the general organization of the body of an acanthocephalan, though the individual organs are subject to much deviation in form in the various genera.

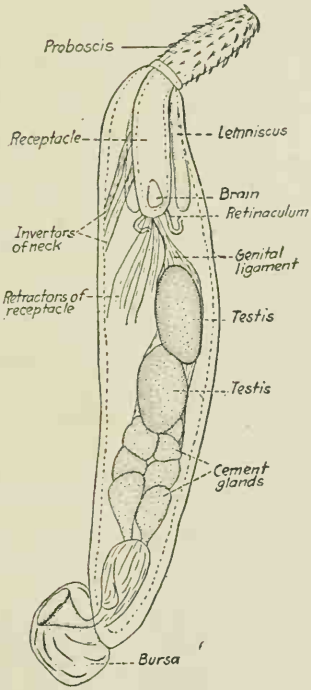

FIG. 1. A male acanthocephalan of the genus Acanthocephalus.

The reference list at the close of this paper includes a number of the more general works which are found useful in the determination of species.

1 (60) Anterior region of body bearing a proboscis upon which hooks or spines are usually arranged in parallel alternating rows..... 2

2 (3) Region between body proper and proboscis (the neck) elongate, cylindrical, except in region adjacent to proboscis where (except in some young individuals) a conspicuous spherical enlargement occurs. **Genus Pomphorhynchus.**

3 (2) Region behind proboscis variously modified but never bearing a spherical enlargement followed by a narrow, elongated neck. 4

4 (41) Body proper never bearing spines. (Demonstration of minute spines on the anterior part of the body in cleared specimens of some genera requires careful adjustment of the light)..... 5

5 (14) Subcuticula bearing a few rounded giant nuclei, prominent in stained whole mounts and in sections and their location frequently recognizable in preserved specimens as minute elevations of the body surface. Wall of proboscis receptacle with but a single muscular layer. Cement gland of males a single syncitial mass with a few giant nuclei. Family Neoechinorhynchidae.... 6

6 (11) Proboscis globular, bearing three circles of hooks..... 7

7 (8) Six hooks in each circle of hooks upon proboscis. **Genus Neoechinorhynchus.**

8 (7) More than six hooks in each circle..... 9

9 (10) Eight hooks in each circle. **Genus Octospinifer.**

10 (9) Twelve hooks in each circle. **Genus Gracilisentis.**

11 (6) Proboscis bearing more than three circles of hooks..... 12

12 (13) Twenty or more circles of hooks. Subcuticular nuclei all in sagittal plane. **Genus Tanaorhamphus.**

13 (12) Eight circles of hooks upon proboscis. Giant nuclei of subcuticula not all in sagittal plane. **Genus Pandosentis.**

14 (5) Subcuticula bearing coarsely branched or finely dendritic nuclei or numerous small nuclei scattered through the subcuticula, but never with rounded giant nuclei..... 15

15 (24) Less than eight cement glands in the male. Proboscis receptacle sac-like, with two muscular layers..... 16

16 (19) Parasitic in birds. Males with three very long tubular cement glands..... 17

17 (18) Proboscis receptacle inserted near middle of the proboscis wall; the portion of the proboscis posterior to the insertion bearing simple, thorn-like spines; anterior to the insertion bearing strong hooks with proteriorly recurved, simple roots. **Genus Centrorhynchus.**

18 (17) Proboscis receptacle inserted at base of a very long cylindrical or clavate proboscis. **Genus Prosthorhynchus.**

19 (16) Males with six cement glands, though of highly variable form.. 20

20 (21) Parasitic in birds. **Genus Plagiorhynchus.**

21 (20) Parasitic in fishes, amphibians, or reptiles..... 22

22 (23) Brain somewhat in front of the posterior extremity of the proboscis receptacle. Retinacula pass through lateral walls of receptacle. Hooks with simple posteriorly recurved roots. **Genus Echinorhynchus.**

23 (22) Brain at posterior extremity of proboscis receptacle. Retinacula pass through posterior extremity of receptacle. In many species lateral or anteriorly directed processes are given off from the main posteriorly directed root. **Genus Acanthocephalus.**

24 (15) With eight cement glands. Parasitic in birds and mammals. 25

25 (26) Proboscis receptacle a closed muscular sac with retractors passing through posterior extremity. Outer layer of receptacle disposed in spiral bands. Hooks small, simple, each with a single simple posteriorly directed root. Parasitic in mammals. Body showing evidences of pseudo-segmentation. **Genus Moniliformis.**

26 (25) Proboscis receptacle a single-walled sac from a cleft in the ventral surface of which the proboscis retractors continue posteriorly through the body cavity. Brain inside the receptacle in region of cleft..... 27

27 (30) Parasitic in birds..... 28

28 (29) Proboscis globular, armed with relatively small number of heavy hooks, roots of some of which bear one or more branches directed anteriorly. **Genus Oligacanthorhynchus.**

29 (28) Anterior region of proboscis provided with strong hooks, basal region (sometimes erroneously termed the neck) with simple spines. Each hook on anterior part of proboscis joins a posteriorly directed root which at the union with the hook is elongated and narrow but expands posteriorly into a much wider flattened, circular, termination. Spines on posterior region of proboscis not always in perfect rows but usually in twice as many longitudinal rows as the hooks on anterior region of the proboscis. **Genus Mediorhynchus.**

30 (27) Parasitic in mammals..... 31

31 (34) Reproductive organs of the male confined to the posterior region of body cavity. Lemnisci filiform, very long..... 32

32 (33) Proboscis provided with a crown of a few circles of strong hooks, crowded so that they may have the appearance of a single row, and behind this crown a region of some length closely set with fine spines. **Genus Gigantorhynchus.**

33 (32) Proboscis with several circles of strong hooks. In intestine of marsupials and edentates. **Genus Hamanniella.**

34 (31) Reproductive organs of male occupy more than one-half of the length of the body cavity. Lemnisci frequently in contact with testes..... 35

35 (38) Cement glands of male arranged in a linear series of four pairs. 36

36 (37) Lemnisci relatively short, flat. Testes considerably distant from cement glands. **Genus Macracanthorhynchus.**

37 (36) Lemnisci relatively long, subcylindrical. Posterior testis not far removed from cement glands. **Genus Echinopardalis.**

38 (35) The eight cement glands not definitely arranged in pairs..... 39

39 (40) Cement glands almost spherical. Lemnisci very long, subcylindrical, more than three-fourths the length of the body cavity. **Genus Oncicola.**

40 (39) Cement glands closely crowded together. **Genus Prosthenorchis.**

41 (4) Body-proper provided with at least a few spines (in some individuals difficult to detect even in cleared specimens)..... 42

42 (45) Spines not limited to anterior region of body, some spines occurring near genital orfice though in some gravid females the genital spines may be lost with the shedding of the copulatory cap.. 43

43 (44) Body just behind the proboscis swollen, closely set with spines and serving for an accessory organ of fixation. Proboscis of medium length. Parasitic as adults in mammals and birds. **Genus Corynosoma.**

44 (43) Anterior region of body slender, spines scattered. Proboscis long. Parasitic in fishes. **Genus Telosentis.**

45 (42) Spines wanting on posterior extremity of body..... 46

46 (47) Body spines limited to a collar composed of four rows encircling the body in the region of the proboscis receptacle. Wall of receptacle but a single muscular layer. Parasitic in fishes. **Genus Quadrigyrus.**

47 (46) Body spines not limited to a few complete circles..... 48

48 (49) Body spines arranged as a collar near the anterior extremity and behind this arranged in 18-23 cross-rows of closely set spines, the rows separated from one another by considerable area devoid of spines, **Genus Serrasentis.**

49 (48) Body spines not arranged in ventral transverse rows widely separated from one another..... 50

50 (51) Parasitic in marine mammals. Spines thickly set as a continuous mantle covering an enlarged anterior extremity of the body-proper. **Genus Bolbosoma.**

51 (50) Parasitic in birds and fishes..... 52

52 (57) Hooks on ventral surface of proboscis distinctly larger and heavier than hooks at corresponding level on dorsal surface.. 53

53 (54) Parasitic in birds. Proboscis usually enlarged near center, spindle-shaped. Anterior region of body usually somewhat enlarged, with its wall of different thickness and different histological composition than the wall of posterior region of body. **Genus Arhythmorhynchus.**

54 (53) Parasitic in fishes. Body cylindrical or tapering gradually, at least without an inflation near anterior extremity, sometimes slightly swollen in region of body spines on ventral surface. Length of proboscis many times the diameter. Body spines ensheathed in prominent cuticular folds..... 55

55 (56) Body spines scattered, especially conspicuous on ventral surface of body. Proboscis hooks protrude at least half their length from surface of proboscis. Brain near middle of proboscis receptacle. **Genus Rhadinorhynchus.**

56 (55) Body spines form an uninterrupted mantle extending backward from anterior extremity of body-proper. Brain near anterior extremity of receptacle. Proboscis hooks protrude but a short distance beyond the heavy cuticula investing the proboscis. **Genus Tegorhynchus.**

57 (52) Proboscis hooks of dorsal and ventral surfaces essentially similar in size. Parasitic in birds. Anterior extremity of body set with fine spines. Neck unarmed..... 58

58 (59) Proboscis ovoid or spherical. Body usually very thick. Anterior extremity of body-proper covered with scattered spines. Between this spined region and the proboscis a long cylindrical neck, frequently retracted within the anterior extremity of the body. In adult females of some species the proboscis is inflated as a large sphere which bears a star-shaped arrangement of radiating rows of hooks at the distal pole. **Genus Filicollis.**

59 (58) Proboscis relatively long, cylindrical or club-shaped, frequently swollen at base; length about twice the diameter. Anterior spined region usually set off from rest of body by a constriction of the body wall. **Genus Polymorphus.**

60 (1) Hook-covered proboscis lacking. Anterior extremity of body enlarged and bearing small pit-like depressions. Beneath the skin around the anus of birds. **Genus Apororhynchus.**

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DEPARTMENT OF METHODS, REVIEWS, ABSTRACTS AND BRIEFER ARTICLES

THE POCKET MICROSCOPE "TAMI"

It is a remarkable sign of progress and a proof of the never resting spirit of the microscope manufacturers, that lately an important innovation has been developed in the construction of a small microscope, the purpose of which is to replace effectively the larger instruments with low or medium magnification.

The idea of constructing such a small, compact and strongly built microscope of excellent optics, at a price which is much lower than those now in use, originated at the factory of M. Hensoldt & Sons, Wetzlar, Germany, who for the last 71 years have been producing high grade optical instruments of military character and for scientific research.

Especially will the botanist, zoologist, entomologist, mineralogist, biologist welcome this new model microscope " T A M I" which measures 4″ in height and 1¾″ in width. The whole instrument is covered up with a solid metal hood; total weight is 15 ounces. Its slender and smooth shape is inviting to carry it in the pocket for outdoor's use.

"T A M I" magnifies 50x and *any degree* up to 225x by simply extending the tube-length, without changes of objectives nor eyepieces. By unscrew-

ing the lower objective system, the magnification ranges from 25x to 112½x.

Illumination is furnished, for transparent objects, by a concave mirror from underneath. The mirror and entire stage is quickly removable and

This illustration shows detached base. The polished glass stage protects the mirror from dust and moisture.

the "TAMI" proper can be placed on extra large, opaque objects, giving the observer a chance to move it all over the surfaces of largest specimens of rock, metal plates, wood, paper, etc., etc.

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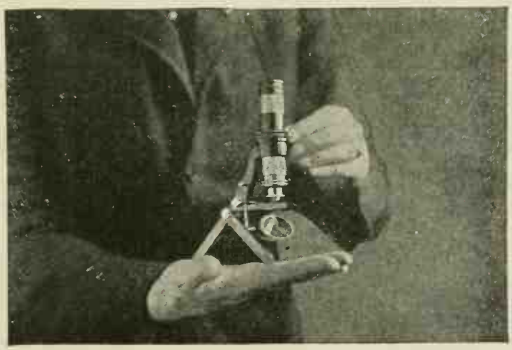
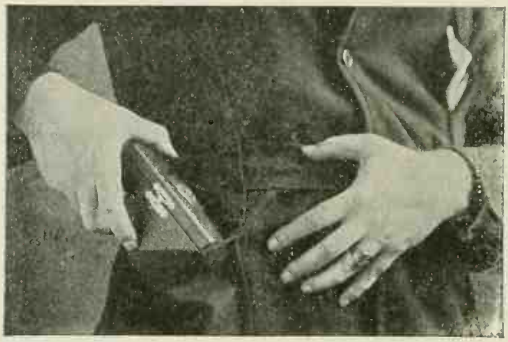
A NEW POCKET MICROSCOPE

A great many American manufacturers produce miniature models which are duplicates of their standard products and which can be used by young folks. One of our largest optical plants has seemingly done a similar thing in producing an extremely small microscope which when folded can be



placed in a leather case pocket size. But this microscope, miniature though it is in size, has adjustments and magnifications equal to many standard models.

The construction allows telescoping of the draw tube and the use of one or both elements of the divisible objective in such manner as to give a wide range of magnifications up to 250x. With these magnifications it can be used in examining a great variety of objects, transparent or opaque, in the laboratory or especially in field work in botany, entomology, mineralogy and general nature study. The magnification is sufficient for clinical examinations, including blood counting, and due to its portability, the instrument may be used at the bedside of the patient.



As mentioned before, in adjustments and operation the pocket microscope resembles the standard models. It is fitted with coarse and fine adjustments which work in easy fashion. The stage is provided with two spring clips which hold the specimen; and the mirror, adjusting in two planes, serves in its regular position under the stage to illuminate transparent specimens. When detached from the mirror bar, it can be placed on a pin at the side of the arm to illuminate opaque specimens.

The instrument is supported by a tripod, the three legs of which fold together and swing back parallel with the tubes and ready to place in the leather covered pocket case, which measures 5 x 2¼ x 2¾ inches.

The microscope weighs 13 oz. and is finished in smooth, durable black.

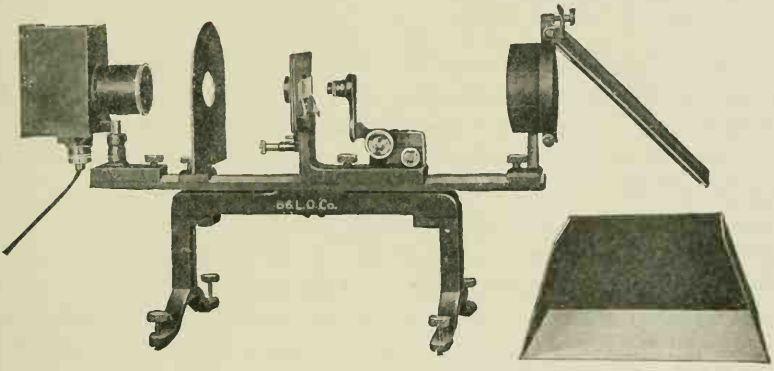
The eyepiece and divisible objective are of high quality and the instrument in every particular is made to the Bausch & Lomb standard. Already the demand has been surprisingly great and with the popularizing of a real microscope, the company predicts a sale beyond estimates.

A NEW DRAWING APPARATUS

As soon as there is a demand for certain research apparatus, it is found that some manufacturer has predicted the need or perhaps has been working along with the user of such equipment and therefore knows the wants of others in research. At any rate, it is certain that in these days apparatus

is not found wanting for any modern use. So this is the case with a new drawing apparatus recently developed which adapts itself to all types of drawing with the special added feature of use in either vertical or horizontal position.

The interchange from horizontal to vertical position is simple and the advantages are great. The optical base, with feet having a spread of 10¾ x 15¾ in., supports the optical bed, carrying the illuminating unit and the microscope, in either position, according to the preference of the user.



When used in a vertical position therefore, the bed is raised perpendicular to the base and is supported by a heavy bracket attached to the base by means of four screws, which can be removed by an ordinary heavy screw driver. The optical bed is fitted on the back (or bottom) with a threaded stud, and two dowel pins on the base hold the bed in alignment when the apparatus is used in a horizontal position. The bed is 25 inches long and graduated so that the parts can be easily relocated when it is desired to duplicate a certain magnification.

The illuminating unit is one of the simplified types with which the 6-volt Mazda lamp is used as the light source.

The condensing lens regularly used is a 60 mm diameter, double convex lens in a spiral focusing mount, which is replaced by the aspheric condenser when the equipment is used for micro projection or photomicrography, because of its superior spherical correction and corresponding increased efficiency.

The microscope used has been especially designed for this equipment, although a standard microscope may be substituted. It has rack and pinion coarse adjustment and fine adjustment which works equally well in either the vertical or horizontal position. The body tube is easily removed to make possible the utlization of the large field, for which such lenses as the Micro Tessars are corrected.

The advantages of this new microscope are obvious. On both sides of the stage holes are drilled for the stage clips, so that the slide, if desired, can be placed on the upper side of the stage when the outfit is used in a vertical position. By placing the slide on the upper or rear side of the stage, the specimen proper is always brought into the same plane with reference to the objective regardless of the variation in thickness of slide, an important factor in reconstruction work where constant magnification must be maintained. The arm carrying the substage condenser has been of special length and makes possible the use of slides of large size, the distance from the supporting post to the center of the stage opening being 2½ in.

When the apparatus is used in a horizontal position for drawing, a reflecting mirror is required to direct the beam downward at 90 to the drawing paper. When the body tube and eyepieces are used, a small mirror can be clamped to the eyepiece tube, but when the low power objectives are being used, a much larger reflecting mirror is necessary to intercept the very wide diverging beam of light. Such a mirror is also suitable with an eyepiece, as the projection distance can be increased by moving the microscope back toward the lamp with a corresponding increase in magnification. The large mirror supplied is first surface type. By removing the reflecting mirror this equipment can be used as a micro projector with very satisfactory results at distances of 10 or 12 feet from the screen. A three-sided metal shield is supplied, which protects the drawing board to a considerable extent from extraneous light.

A very unique camera attachment can be secured for use in conjunction with this apparatus when photographs at moderate magnifications are to be made. It consists of a sheet metal box attached to the base by a bracket and two thumb screws, the bottom of the attachment resting upon the table. The plate holder and focusing screen are at the bottom of this box. The image is observed on the white focusing screen through the opening at the top of the camera box; a light excluding metal slide closes up this opening while the exposure is being made.

The many uses to which this apparatus can be put make it extremely useful for general laboratory work.

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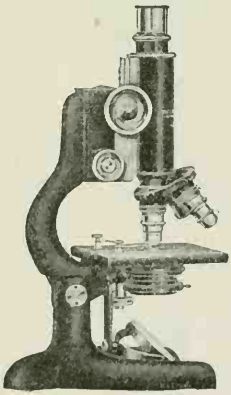

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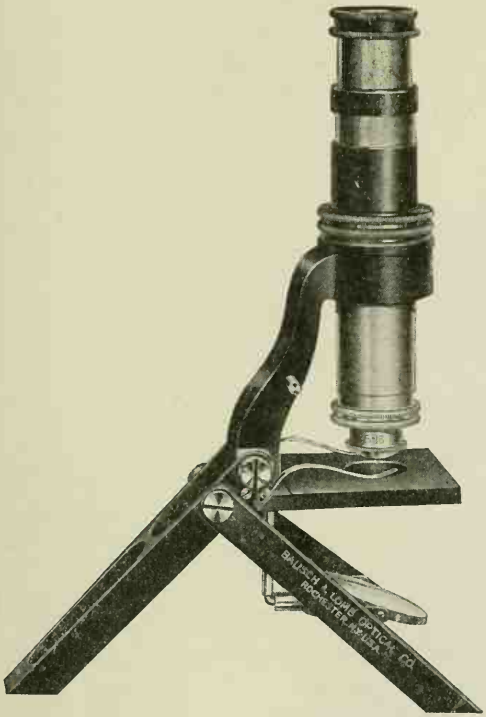

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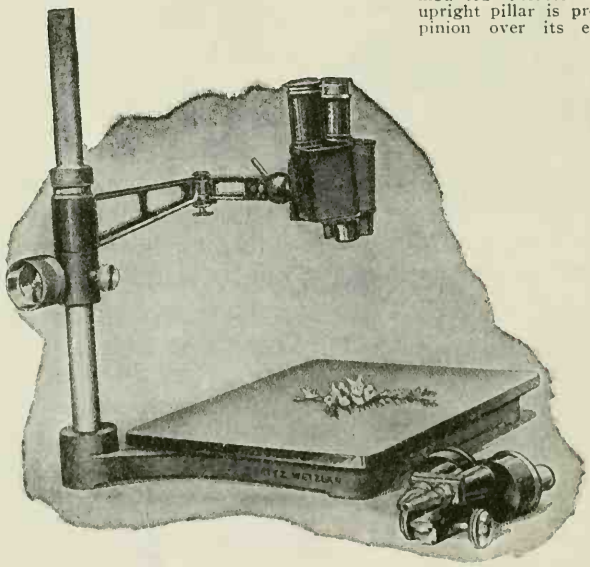

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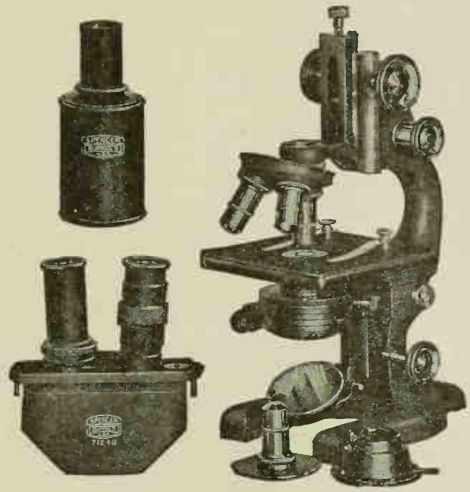

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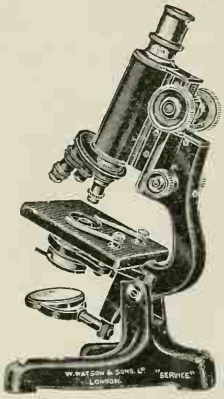

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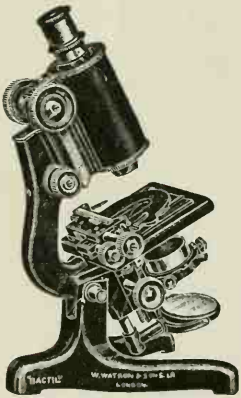

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